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Don't move!

FOR many years commissions, committees, working parties and individuals have pondered the problem of the scientific civil servant and his or her career prospects. In the last year or two much of this thinking has been fuelled by concern (at least by those on the outside) that the civil servant, with a generally securer job and an excellent pension scheme, has also been paid considerably better than his counterpart elsewhere. But long before this became an issue many thinking people, by no means all outsiders, were worried that the scientific civil service lacked either enough right openings for mature scientists or any means of goading such scientists not to sit and fester within the service. The Bondi Task Force which reported in 1974 was the most recent group to look at the problem, and came up with proposals to increase mobility into and out of the service.

A small unit was set up in the Civil Service Department in 1972, initially to work with the Bondi Task Force but eventually to proceed under its own steam in stimulating mobility. Unfortunately, its terms of reference were limited. Many believe that the best way to stimulate a scientist to give of his best and the best way to encourage an organisation to make the best use of its manpower is for the employer to make it quite clear to the employee at the outset that no one can be promised forty years of continuous satisfying employment. Under these circumstances there could be a valuable trade at all levels of people into and out of the civil service.

But this is not to be—and at times like the present, when projects are being trimmed or cancelled, note how the civil and public servants remain while industrial contractors have to cope with redundancies. The unit which was established was not given the job of helping people to make major switches in career; it was given the much more timid mission of stimulating two-year (typically) secondments into and out of the service—but with a guaranteed return ticket. And even this relatively unadventurous project has hardly met with waves of enthusiasm, as a recently circulated progress report of the Scientists Interchange Unit makes clear.

It is difficult to estimate exactly how many within the service might in one circumstance or another be candidates for secondment, or on the other hand how many

openings might arise which could be filled on a short-term basis by outsiders. But there are 2,300 Principal Scientific Officers in the service, and if we include other grades above and below this level and some in the analogous technical officer grades there might be 5,000 who should at least know about the scheme.

Yet in the last three years only 23 made an approach to the unit, and of these only five could be found places outside—four in industry, one in a research council. In the same period 20 secondments were made into the service—nine from industry, ten from universities and one from a research council. It is only fair to add that government departments sometimes arrange their own secondments without going through the unit, and in the same three years 18 did an outward move (though less than half into industry) and nine an inward move (two from industry).

The picture, as the unit admits, is disappointing. There seems little enthusiasm for tasting another job even with all the safety of one's own job being kept warm. It would be easy to conclude that the average British scientist is feeble-minded and unenterprising. That may indeed be true, but it should be made clear that the present scheme is actually a much more tricky one to make successful, and maybe a much less attractive one than a one-way ticket scheme. Skills have to be closely matched, employers have to be convinced that loss of employees for two years is in their interests, petty jealousies over salaries have to be overcome, temporary accommodation and schooling has to be found if a move is necessary, the briefing period may be a significant fraction of the total time available and so on.

Permanent moves could not be more difficult and indeed might be very much easier for all concerned. Certainly without them the same old accusations will continue to be thrown at the civil service, and scientists will have achieved little on their side to facilitate the more open style of government for which many yearn and which requires more flow into and out of government establishments. The Bondi Task Force should reconvene to consider, even in a time of gloomy economic prognostications, whether it cannot push for more than just secondments. □



Nuclear power, public interest and the professional

In the case of major and complex technological decisions in our time, such as that on nuclear power, it is sometimes said that the public is unable to make a full evaluation on its own and needs to trust some established professional and political mechanism instead. Jan M. Döderlein of the Institutt for Atomenergi, Norway, comments on the roles of the professionals and the nuclear critics.

NUCLEAR technology came to maturity at a time when attention was rightly focused more than before on the environmental aspects of industrial and energy production. As a result, in many countries nuclear stations are the only type of electric power plant for which detailed safety and environmental evaluations by government agencies are required, and where these evaluations are open to direct public participation. This has helped to create a public controversy over nuclear power, a controversy which appears in large measure to have spread from the United States. It presently focuses on three main topics: nuclear reactor accidents, the handling of radioactive waste, and the increased risks of proliferation of nuclear weapons (and therefore presumably of nuclear wars) from an expanding peaceful use of nuclear power.

Probably no technological decision in the history of mankind has been the subject of so many detailed studies, so much open discussion and such broad public participation. Amongst it all, some nuclear critics have given prominence to ethical aspects of the nuclear decision. This is justifiable to a certain extent since the application of nuclear power—indeed of any power—raises ethical questions. To carry this view to extremes in the way it is sometimes done, however, represents a vain attempt to set aside fundamental laws of nature and society.

One aspect of such ethical considerations is seldom if ever mentioned. The number of publications on astrology, on transcendental meditation, on nirvana and similar subjects is increasing sharply. Among the younger generations, and in some leading cultural and political groups, increasing numbers of people are seeking mystical or irrational escapes from many realities of modern society. There are thus clear indications that superstition, mysticism and emotionalism are on the upsurge.

Another symptom of this surge of emotionalism is the apparent tendency among sensitive and highly responsible citizens to shoulder the burden of guilt associated with the expansion of modern society. With some leading personalities in politics, industry and science, this burden is transformed into a desire to steer the development of

society away from perceived future cataclysms. Sometimes the bases for assuming such cataclysms are world models created by scientists. These provide some predictions of a near disastrous future for our society with the combined authority of science and leading personalities in society. Such an authority is merited neither by the quality of the world models nor by their basic assumptions.

One of the consequences of irrationalism and emotionalism is a desire for simplification of life and for a low energy society, a desire which is reinforced by "scientific" doomsday prophecies. Another is a strong sentiment against the people who are working for the application of technology within society, which erodes confidence in expertise. Nuclear power is not a simple technology; it can produce prodigious amounts of energy; emotionally it can be construed as having a certain "doomsday character"; and it requires qualified expertise. Nuclear power can therefore be seen as the antithesis of irrationalism, a view which easily creates a strong, emotionally based anti-nuclear attitude.

A natural outlet

The nuclear controversy is therefore a natural outlet for many irrational needs in our society, not least important of which are the irrational needs of many scientists, technologists and experts. These views are supported and broadened by fundamental observations made in another context by the epistemologist Karl Popper. He stated that, when threatened,

"the rationalist attitude to social and economic questions could hardly resist when historicist prophecy and oracular irrationalism made a frontal attack on it. This is why the conflict between rationalism and irrationalism has become the most important intellectual and perhaps even moral issue of our time".

One perspective on the nuclear debate, then, is that our dominant problem may be that of fighting emotionalism and irrationalism. This is not to use the words "irrational" and "emotional" in any derogatory sense. The fight is not against emotions or the irrational. The issue is the application of irrationalism and emotionalism in attacking major problems of our real, physical world.

The emotional and ethical aspects of nuclear power are trumpeted particularly by two groups of critics having the common denominator of emotionalism. One group consists of people for whom nuclear power is a vehicle of self-realisation. They play out some of their inner and emotional needs by taking an active part in the nuclear controversy, and the factual nuclear issues seem to play a secondary role. The other emotional group of nuclear critics is composed of professional and amateur politicians, notably leftist groups, anarchists, some populists and some environmentalists. Nuclear questions, real or imagined, are a means by which they further their own political goals, sometimes clandestinely, sometimes openly. A very different group of critics, the opportunists and malcontents, is cynically achieving fame and prominence by going into the nuclear debate, writing in the newspapers, giving lectures, going on television and so on. Needless to say, similar people are also found in the so-called nuclear community.

Characteristic shared

All three groups of nuclear critics share one characteristic: they seem to accept authoritarianism as a road to knowledge, often to the exclusion of other sources of knowledge. They rarely support their assertions with reasoned arguments or with facts, and they consistently invoke the opinions of some distinguished scientists, some Nobel-prize winners or some professional societies, ignoring others who disagree with their views. By confusing technical facts which can never be argued qualitatively, with value-judgments which can always be argued, the critics have in some countries succeeded in misleading sections of the public, causing them to believe that everything is open to argument and that the experts are confused. The truth is that the experts are not confused in their own field of expertise. Outside their own field they are not experts.

In the past two years we have seen a plethora of manifestos and statements for or against nuclear power by Nobel prize winners, by groups of scientists and by various professional societies. In connection with the statements, the eminent or special qualifications of the participants are always emphasised. While possibly not without merit, such statements should be viewed very critically. Practically without exception the participants in the anti-nuclear manifestos have no technical background in fields relevant to nuclear technology. Against the participants in pro-nuclear

manifestos, this criticism is less relevant.

One criticism is valid against all manifestos on nuclear power. The scale and timing of the introduction of nuclear power is a major and complex decision, resting on technical, economical, political and other social considerations. In a democratic society such decisions are not left to technological experts but to elected political officials. A professional using his specialist background in support of personal views on social questions tries to carry his professional authority over into fields where he should no longer have such authority, into fields where his opinions should be weighted on the "one man, one vote" principle.

Members of some professions believe they have more specialised knowledge, and that their political conclusions are based on sounder analysis of the evidence than the public. If one expects the public to have confidence in the role of professionals in decisions, the opinions of highly regarded experts within their chosen profession must be accorded a certain authority. But it is equally important not to support a carry-over of professional authority to political and ethical questions, a carry-over which may indicate a certain intellectual arrogance.

Assessing the 'facts'

Many "facts" in the nuclear controversy are to all appearances hotly contested. Some of these "facts" are not really facts, and deserve to be independently and critically evaluated by professionals and non-professionals alike. Among such "facts" in the nuclear debate are some aspects concerning the long term effects of radioactive waste, and the possible connections between nuclear power and proliferation of nuclear weapons.

Among the often repeated and confusing statements on radioactive waste are the suggestions that in this waste man produces materials that (uniquely) remain toxic for times much longer than we have had experience with, and that in nuclear reactors we have created materials that have never existed before.

Certain elements created in substantial quantities by nuclear power, for example plutonium (but not waste proper), remain toxic for times longer than the period of human civilisation. No industrial scale method for disposing permanently of radioactive waste is in use today. Deciding on appropriate handling of this waste is an important and difficult question which many countries are addressing. At a pilot plant level, however, there are solutions to the *technological* problems. Moreover the long life of

radioactive waste should not be judged in isolation; industry including power production from oil and coal gives uncontrolled releases to the environment of substances which remain toxic for infinite times; civilisation is accustomed to living with poisonous materials that remain toxic for times longer than the life of radioactive substances. For civilisation as we know it there is thus only a choice of *which* toxic materials we wish to handle.

With nuclear power man has not created any materials new to earth. Specifically, fission products and plutonium are naturally occurring elements, albeit in small concentrations. The ethical objection to nuclear power sometimes made on the basis of creation of "new" elements therefore has no foundation in fact.

The question of a possible connection between nuclear power and proliferation of nuclear weapons really has two separate aspects: one concerning sub-national groups using atomic explosives for terrorist purposes, the other concerning a possible increase in the number of sovereign nations with access to nuclear weapons. The understandable but unfortunate preoccupation of the mass media and the public with the matter of nuclear terrorism is distracting our attention from the far more important question of possible proliferation of atomic weapons beyond the present weapons states.

It is misleading to ask whether the spread of nuclear power inevitably leads to the spread of nuclear weapons. The peaceful uses of nuclear technology are now widespread; there are substantial quantities of peaceful and military fissile materials. The relevant question is whether a halt to the expansion of nuclear power, or to all use of nuclear power, will substantially reduce the potential for wars and the risk to the public from wars. Clearly it would not be meaningful to curtail the use of nuclear power for this reason without at the same time dismantling the many smaller reactors used for medical purposes, isotope production and research in most countries of the world. This aspect takes on added importance when one considers the substantially more complex operations required for using a power station rather than a small reactor for plutonium production.

Technological developments are likely to complicate further our questions about weapons proliferation. Within a few years there will be available, worldwide, several new techniques for enriching uranium. All of those being researched today are potentially simpler technologically than the present diffusion technique, and all of them can more easily be applied on a small scale. Ensuring peaceful uses

only of these techniques may be of more importance than the question of plutonium safeguards.

Unfortunately, nuclear proliferation is only part of a more fundamental and more serious contemporary question: can anything short of actual war stop a determined sovereign nation from getting primitive but usable versions of any kind of contemporary weapons technology—nuclear, bacterial, chemical or any other? Be that as it may, broad agreement could probably be obtained to curtail development of nuclear power if rational arguments could support an assertion that stopping peaceful uses of nuclear energy would reduce, if only marginally, the risk to the public from war and acts of terrorism. But reasonable, rational and valid arguments to this effect have not been put forward so far.

Reducing the risks

On this basis, expending efforts to stop the use of nuclear power does not seem to be a credible and useful way of reducing the risks of nuclear wars. Our most responsible and efficient attack on the nuclear proliferation problem would seem to be to channel our efforts into support for the UN and the nuclear supplier nations in their non-proliferation efforts; into the expansion of international safeguards inspection, including inspection of arrangements to protect physically all fissile material; into support for all practicable means of making the time span and the effort involved in converting in-reactor plutonium production to a workable nuclear weapon as large as possible; and last but not least into the problems created by the availability of new uranium enrichment techniques.

All human actions entail some unforeseeable consequences. Plans for action cannot be based solely on factual proofs and logical deductions; they must finally rest on political decisions made in the face of uncertainty, a point which gives a perspective on the place of technological facts in a world of values and emotions. Clearly, a number of irrational, emotional and ethical factors may be and should be of importance in a choice of power plants for the production of electricity. But an evaluation of *rational and quantifiable* factors tells us how many lives, which environmental improvements and what economic advantages we have to sacrifice in order to satisfy such emotional demands. In defining the role of the professional and in protecting the public interest, the importance of emotional factors in the nuclear controversy must be admitted; but as many problems as possible should be decided on a reasoned, factual and rational basis. □

No university without research

Gillian Boucher looks at how the Open University copes with the problem of conducting research

IT would be a forgivable mistake for anyone outside Britain's Open University to imagine that its sole function was to provide a university education by post: virtually all publicity has concentrated on its innovative and immensely popular courses. Even within the university it has sometimes been difficult to remember that there could be any aim in life beyond producing the next course unit. But the Open University's charter is utterly normal in allowing and requiring staff to undertake research, and recently, with the worst of the growing pains over, there has been a reassertion of the vital need for research to take its proper place alongside teaching.

Of course this view has always existed: most open university lecturers like research too much to forego it willingly and believe that an institution that simply packages information does not deserve to be called a university. But initially many people just did not have time for research. There are some who have gravitated towards the Open University because they get more satisfaction from teaching than from research. And there is a strong current of opinion in the university administration that says that research is a prize for the good boys who have written their courses. In some faculties there is even a feeling that science in general and scientific research in particular are expensive luxuries. But in spite of the difficulties the university has never been devoid of research.

Steven Rose and Ian Gass, professors respectively of Biology and Earth Sciences, managed to bring a nucleus of staff with them when they moved to Milton Keynes and have been gradually stepping up their activity ever since. The Department of Earth Sciences now produces more papers than any other British Earth Sciences Department except the Cambridge Department of Geophysics. The small staff (10 lecturers in the Biology Department) means that it is not feasible for everybody to work independently: intellectual companionship is only gained by joining a more or less interdisciplinary group. The obvious example is Rose's Brain Research Group, which involves five lectures and 15 others and is by far the largest research effort in the Biology Department. Another group, the Energy Group, was formed spontaneously during the preparation of a course on materials. Now the scientists, engineers, architects and economists in the group are studying

problems as various as the British gas industry and the development of a house heating system based on the same principles as the refrigerator cooling system.

Funding a problem

One of the programme's great advantages, of course, is cheapness. Funding is obviously a problem for an institution trying to grow at a time of economic crisis and government lack of sympathy with the universities. For an established group funding from the university and from outside sources is adequate if not princely. But for others there are difficulties: the university's approach is to fund a project initially but to expect it to attract outside funds to continue if it is any good; if the strings of published papers required by the SRC have not been written, the project is likely to founder.

Working conditions are not very comfortable and one of the problems in creating a stimulating academic environment is that people tend to spend a lot of their time at home writing course units. The permanent science building originally scheduled for 1974 will be ready in 1977 at the earliest and even this will not hold all staff: the biophysics and other groups temporarily housed in Oxford will stay there for at least another five years. That is not an unmitigated disaster—among the charms that Oxford possesses and Milton Keynes lacks are first-rate libraries and the ease with which temporary self-funded American

researchers may be attracted.

The Open University has always had research students—both internal students at Milton Keynes and Oxford and external students for whom the university finds an external supervisor near home. Many external students are technicians or hospital scientists and must of course have access to the equipment they need. Trying to do a PhD and a job at the same time sometimes causes insuperable problems: in many areas knowledge is increasing almost too fast for a part-time researcher to keep up, let alone make his own contribution.

At present higher degree students with Open University first degrees are rare, simply because so few Open University graduates have yet been produced. When they do start demanding higher degrees in large numbers there will be difficulties. The open university student, though probably more resourceful than the average British undergraduate, is not used to reading much outside his course text or doing much practical work. In many ways the Open University degree is closer to the American than to the conventional British one. American PhD students usually spend a couple of years doing course work before beginning research but the Open University offers no postgraduate courses. The prospects are not good: the undergraduate courses are voracious and the Department of Education and Science has not responded warmly to the suggestion that it might earmark certain sums for postgraduate courses. In terms of mere numbers this is understandable—at present there are 50,000 undergraduates and 400 graduate students—but the development of postgraduate courses will be valuable. □

Profits in academia

One way the Open University is hoping to cushion itself from the uncertainties of government funding is by setting up a company to market its educational materials. Marketing as a part of the university administration is as old as the university itself but it is hoped that Open University Educational Enterprises Ltd will greatly expand the business.

All is still in the air, though, while the university discusses the project with the Department of Education and Science which, after the resounding failure of a similar venture, British Museum Publications Ltd, is somewhat nervous of letting public funds be used as risk capital. The OU, however, is confident that the go-ahead will come in time for the company to start business on January 1 as planned. Large amounts of capital are not required as some of the present marketing division's profits are ploughed back into marketing. According to the university the talks are mainly to thrash out the right relationship of the new company to the university and to make sure that it is properly structured.

From the university's point of view, a company owned by the university has several advantages over a division of the university administration, among them the freedom the company would have to offer salesmen a suitably attractive salary instead of having to pay them on academic administration scales. The company may also clarify the copyright position—at present although the written word is the main teaching medium in the Open University the academics hold the copyright on their works as in any other university.

More than half the income from the sale of the Open University's books, tapes, films and cheap scientific equipment comes from abroad. The university feels there is plenty of room for the marketing company to expand: the marketing division cannot deal as well as it would like even with its present markets. If the new company succeeds, as is hoped, in increasing turnover from the present £700,000 a year to £1 million by 1980, it will be contributing a significant supplement to the £12 million government grant.

USA

Seeking Senate surgery

A radical overhaul of the Senate Committee system is being discussed in Washington. Colin Norman reports on the implications for scientific affairs

AN entertaining display of political horsetrading, perhaps culminating in a good public scrap, is about to unfold on Capitol Hill. For the first time in more than a quarter of a century members of the US Senate are being forced, somewhat reluctantly, to take a close look at that august body's confused and inefficient but immensely important system of legislative committees. Some sweeping reforms, which could wipe out entire committees and with them the power bases of a number of influential Senators, are under discussion.

The proposed reforms were put forward a few weeks ago by a special select committee. But because public attention was diverted by the marginally more interesting spectacle of the Presidential election and a third of the Senate were preoccupied with getting themselves elected, few people took much notice. The Senate is expected to take up the proposals soon after it reconvenes in January, however, and so they are now becoming a topic of intense interest on Capitol Hill.

There are a good many political obstacles to be overcome before the proposed reforms are accepted, but if they are ever implemented they will have a substantial effect on the way the Senate handles legislation on scientific and technological matters. For a start, responsibility for energy research and development would be placed in a single committee, the jurisdiction of the present Commerce Committee would be expanded to take in most non-military science and technology programmes, and the Joint Committee on Atomic Energy, which has charted the course of the United States' nuclear policy for some 30 years, would be abolished.

Overall, the proposals would reduce the numbers of Senate committees from 31 to 15, doing away with a variety of select, standing and joint committees, and realigning the jurisdictions of most of the remainder. The Senate's present 174 subcommittees would be cut down to about 100, and no Senator would be able to sit on more than 8 subcommittees. In short, the proposed reforms represent some pretty radical surgery, and they would bring about the first real changes in the Senate committee system for 30 years.

Even a cursory look at the system confirms the need for a knife. The Senate's committees are so sprawling

and their jurisdictions so confused that it is frequently difficult to tell which committee has authority over individual bills. Take energy research and development, for example. The Joint Committee is deep into one aspect of energy, so is the Interior Committee, the Public Works Committee, the Aeronautical and Space Science Committee, the Commerce Committee and the Labor and Public Welfare Committee. A couple of years ago, when a relatively non-controversial solar energy bill was passed by the House, no fewer than four Senate Committees claimed jurisdiction and it had to be approved by all of them before finally reaching the Senate floor.

But, though the need for reform is obvious, it is going to be a tough battle to get anything done. The problem is that Congressional committees, and to a lesser extent subcommittees, represent overlapping fiefdoms headed by powerful, and frequently elderly, chairmen whose authority has taken years to acquire. The chairmanship usually goes to the longest-serving member of the majority party on each committee, and with it comes a good deal of power to appoint committee staff, and to shape or even obstruct the passage of legislation. Thus any attempt at reform must first overcome the fact that a lot of powerful people are going to resist having their authority altered or even removed.

The House went through a mild reorganisation a couple of years ago in a move which gave the House Committee on Science and Technology a good deal more power. But those changes were only made after a couple of years' of intense negotiations and six days of shrill public debate. The Senate reforms may be equally difficult—moves are already afoot to try to shunt them aside by simply referring the whole matter to the Senate Rules Committee for further, prolonged study—but their proponents point out that conditions for change are more favourable now than at any time in the past several years. For one thing, there will be seventeen new faces in the Senate next January—a much higher turnover than usual. And for another, the chairmen of four committees threatened with the axe were either defeated or retired.

The following are the major changes proposed by the select committee in committees concerned with science and technology. It should be noted that the proposals would leave essentially untouched the real powerhouses, such as the Appropriations Committee, the

Armed Services Committee, the Finance Committee and the Judiciary Committee. Thus, some of the most powerful Senators won't be too badly affected, and appropriations for government agencies will continue to be handled by the same appropriations committees and subcommittees.

- It has been proposed that responsibility for non-military research and development policy should now fall under the present Commerce Committee, whose authority would be greatly expanded and whose name would be changed to the Committee on Commerce, Science and Transportation. The new committee would be responsible for keeping its eye on the activities of agencies such as the National Science Foundation, the Office of Science and Technology Policy, the National Aeronautics and Space Administration and the various agencies of the Departments of Commerce and Transportation. It would be the chief committee for federal research activities other than military, biomedical and energy programmes. If the proposed changes are accepted, they would cause the demise of the Aeronautical and Space Science Committee whose chairman, Frank Moss, was defeated at the polls in Utah.

- The change would also mean that Senator Kennedy would lose the authority he now holds over the National Science Foundation through his chairmanship of the NSF subcommittee of the Committee on Labor and Public Welfare. That committee would be expanded under the proposed reforms to become a Committee on Human Resources, and Kennedy is said to be negotiating to have NSF affairs consigned to it. It wouldn't be the most logical place for NSF, but at least Kennedy would be able to retain his jurisdiction.

The proposed Human Resources Committee would be responsible for, among other things, health and biomedical research, which means that Kennedy would retain much of his authority over the programmes of the National Institutes of Health.

- As for energy research and development, the select committee has proposed that the responsibilities of the present Interior Committee, headed by Senator Henry Jackson, should be expanded to include most federal energy development efforts. Renamed the Committee on Energy and Natural Resources, the new body would pick up responsibilities from the Joint Committee on Atomic Energy and a number of other committees. The proposal would put the knife into the back of the Joint Committee, the once-powerful unit which has shaped nuclear policy

since the second World War.

The Joint Committee is in trouble for other reasons, too. Next month, when Democratic members of the House meet to discuss the political agenda there, two nuclear critics, Clarence Long of Maryland and Jonathan Bingham of New York, will propose that the committee be scrapped and that some of its functions be transferred to the House Science and Technology Committee, among others. Thus, the Joint Committee is under combined attack in both the House and the Senate, and its chances of surviving are generally considered to be less than even. One point going against the committee is that five of its nine members, including the chairman, Senator John Pastore of Rhode Island, either retire at the end of this year or were defeated at the polls. Moreover, two of the most powerful members of the Joint Committee, Senator Jackson and Representative Mike McCormack, would stand to inherit some of its

authority in their own committees, so they probably wouldn't be too sorry to see the Joint Committee disappear. It should be noted that the Joint Committee has been consistently pro-nuclear, and its demise would be welcomed by anti-nuclear critics.

● Another major Senate change proposed by the select committee is the creation of a new committee on Environment and Public Works, founded mostly on the present Public Works Committee headed by Senator Jennings Randolph. It would be responsible for environmental programmes together with nuclear regulation, which would mean that responsibilities for developing and regulating nuclear power would be divided between separate committees.

The proposed reforms are expected to be one of the first items of business when the Senate reconvenes early in January, but negotiations are already under way behind the scenes, as Senators who stand to lose some of

their jurisdiction are striving to hang on to their authority.

It is quite possible that the opposition will be too strong and that the proposals will be quietly killed off or severely watered down by consigning them to the Rules Committee for further study. But at least the proponents of the reforms are preparing to put up a spirited fight. Senator Adlai Stevenson, the chairman of the select committee, is said to be threatening to introduce a resolution blocking the appointment of new senators to committees and the appointment of new committee chairmen if his proposals are simply shunted aside. Such a resolution could tie up the business of the Senate for a long time if it were approved. A more likely development is that differences will be settled by behind-the-scenes negotiations in the next few weeks, and a compromise reform plan will be worked out in conjunction with the Rules Committee. □

Genetic manipulation: enter the environmentalists

LAWYERS for two environmental organisations have filed a formal petition with the Department of Health, Education and Welfare (HEW) requesting extensive public hearings and the development of legally binding regulations to control all recombinant DNA experiments in the United States. It is the first time that environmentalist groups have formally entered the swirling dispute over the risks and benefits associated with recombinant DNA, and the petition is probably only the opening shot in what could develop into a lengthy legal fight.

Filed by the Environmental Defense Fund and the Natural Resources Defense Council, the petition has been endorsed by Robert L. Sinsheimer, chairman of the Division of Biology at California Institute of Technology. Sinsheimer, a leading critic of recently-issued safety guidelines governing recombinant DNA research supported by the National Institutes of Health (NIH), sent a letter to HEW along with the petition.

The petition requests two actions by HEW. First, public hearings should be held to allow interested parties to state their case and to have their views taken into account; and then HEW should develop binding regulations to control all recombinant DNA experiments in the United States. In the meantime, the petition asks HEW to extend the voluntary NIH guidelines to cover experiments supported by other agencies and by

non-government bodies.

The petition is clearly motivated by the belief that the NIH guidelines are inadequate to control the potential hazards associated with recombinant DNA research. It states that the guidelines "are the product of the deliberations of scientists who are now conducting recombinant DNA research", and argues that "little discussion was devoted to whether or not these experiments ought to be performed *at all*, even though the question was raised both by concerned laymen and by prominent scientists".

The petition also points out that the chief drawback in the guidelines is that they formally apply only to research supported by NIH, leaving industrial research essentially unregulated. Moreover, there is no requirement for federal monitoring to ensure that the guidelines are being followed. Those drawbacks are also bothering some state and local officials, in New York State for example.

The petition will probably be turned down on the grounds that HEW lacks authority to tell other agencies or private industry what to do, but the environmental groups are likely to press their case either in the courts or on Capitol Hill. Senator Edward Kennedy has already said that he may consider introducing legislation to make the NIH guidelines binding on everybody who wants to conduct recombinant DNA studies.

Meanwhile, an inter-agency task

force has been established in Washington to discuss ways in which the NIH guidelines can be extended to other federal agencies. According to one official concerned with the task force, it will also discuss the possibility of establishing a monitoring procedure to ensure that the guidelines are followed, and it will also look into what industry is doing and whether the federal government could (or should) regulate industrial recombinant DNA experiments. One possible outcome of the task force's deliberations is that the NIH guidelines may be made into legally binding regulations covering all agencies and industry as well. If so, that would accomplish some of the petition's demands.

The task force is headed by Dr Fredrickson, and consists of representatives of other federal agencies. It meets in private since it does not fall under the terms of the advisory committee act, and it is expected to come up with some recommendations by mid-January.

If it does recommend the adoption of legally binding regulations and a monitoring procedure, a key question would be which agency should take responsibility for enforcing them. NIH officials are anxious not to be placed in the position of both supporting and regulating the research, and the task may therefore fall to a regulatory agency such as the Occupational Safety and Health Administration.

USSR

Space programme: accentuating the cooperative

The Soviet Union will celebrate the twentieth anniversary of Sputnik-1 next year at the same time as the diamond jubilee of the October Revolution. Vera Rich reports

THE launching of Sputnik-1 on October 4, 1957, inaugurated a whole series of Soviet "firsts" in space, frequently co-ordinated (so far as the state of the art permitted) with some notable anniversary. The centenary of Lenin's birth was marked in 1971, for example, by the launch of the first orbiting space-station, Salyut 1; and it is entirely possible that the Soviet space programme is planning some equally prestigious "spectacular" for the sixtieth anniversary of the Revolution. The current emphasis, however, suggests that the year will be marked first and foremost by an extension of "fraternal cooperation" in space between the Soviet Union and her Comecon partners.

This trend is not a cover-up for the recent set-back to the Soviet space programme provided by the aborted Soyuz 23 mission when it failed to link up with the Salyut 5 station. On the contrary, it is perhaps best exemplified by the joint communique issued by Soviet Academician Boris Petrov and GDR Academician Karl Gröte, after the previous highly successful Soyuz flight. Soyuz 22 had carried a "multi-spectrum" camera from the Karl Zeiss (Jena) works, and was heralded as a new phase in Comecon cooperation in space. The same theme was enthusiastically repeated by the Soviet and Comecon media.

The presence of foreign equipment aboard a Soviet spacecraft is not new; French instruments were carried on Lunokhod 2 and by the Mars 5, 6, and 7 probes. Comecon has had its own space programme, Interkosmos, since 1967, and has launched 16 orbital satellites and 4 high altitude rockets. The Soviet Union supplies the rocketry for all Interkosmos launches, while her Comecon partners supply certain instruments, so the use of such instruments in a formally Soviet mission might seem a fairly predictable development. Why, then, was it greeted with such acclaim?

Propaganda plays a certain part: the current Comecon five-year plan, while temporarily soft-peddalling the final goal of total economic integration, is concentrating on a short term drive

(15-20 years) towards integration in certain significant areas, notably sophisticated instrumentation. The Petrov-Gröte communication clearly refers to this policy, stating that East German participation in Soyuz 22 was "part of the process of Socialist integration in one of the newest regions of contemporary science and technology".

The concept of an integrated science policy is, however, meeting with opposition from certain research bodies and institutions in the member countries of Comecon, particularly where, as in Poland, there is a long native scientific tradition. Such bodies have been openly accused of "autarchic" (that is, nationalist) tendencies, necessitating the intervention of Party and

Government. Emphasis on cooperation in such a prestigious field as space research might well be interpreted as simply a tactical measure to offset in the mind of the public the effect of this academic discontent.

Earlier this year it was announced that Interkosmos would start a manned space programme with multinational crews. The first launches are scheduled for 1977 and the cosmonauts are already in training. It would seem, however, that each crew is to include at least one Soviet cosmonaut. Since the Soyuz 11 tragedy, though, the Soyuz missions have used a two-man crew, instead of the previous three—a change apparently associated with the decision that the crew should wear space-suits during re-entry as a safety measure. If the current pattern continues, it will take at least eight flights for each country to get one representative in space.

Expeditions: resources rationally used?

The rational use of natural resources in the Soviet economy is a major target of the current five-year plan. It is also a dogma which can be invoked to justify most things. That includes geological and geographical expeditions, where the data obtained can be interpreted to relate to the needs of prospecting, oceanography, climatology and other fields of practical significance. In the case of the expeditions of summer 1976, the range is from the immediately practical to the extremely remote in terms of the economy's needs.

On the one hand, there was massive Soviet participation in the 200-strong joint Comecon expedition to Mongolia, which worked within a surveying programme aimed at ensuring 10-20 years' supply of fuel and material resources for the Comecon bloc. During this first field season some 7,000 km² was systematically surveyed, and several outcrops of "valuable raw materials" (so far unspecified) were located. The expedition is a long-term project; to facilitate its work in future seasons a special base is to be established next year on the outskirts of Ulan-Bator, the Mongolian capital, with administrative and living accommodation as well as such services as shops and a kindergarten.

Although even the most cautious planners could hardly doubt the practical value of the Mongolian expedi-

tion, they might feel a little different about the other major long-term project inaugurated this summer. This is the scheme to sink a 16-km borehole at Saatly in the Murganskaya steppe. This area is a "geological anomaly", where the crust is significantly thinner. In 1970, a pilot borehole was sunk to a depth of 6,240 m. If successful the new project will allow material to be recovered from the upper mantle. The theoretical value of such specimens would be immense; the most practical outcome of the expedition may well be the experience which the Soviet oil industry will gain in sophisticated drilling techniques.

One of the most practically-orientated expeditions this summer was that of the Siberian Energy Institute of the Soviet Academy of Sciences, which set out to survey possible power resources in the construction area of the new Baikal-Amur Mainline railway. The Baikal area has long been famous for its geographical and ecological riddles; this expedition discovered another, a complex of "classical mountain glaciers" at a relatively low altitude and unexpectedly far south. The plans for the railway have from the beginning incorporated a number of conservation measures aimed at preserving the unique Baikal habitat. The presence of these glaciers "in direct proximity of the line" is causing the relevant experts considerable concern.

The head of the cosmonaut training programme, Lieutenant General Vladimir Shatalov, has spoken in terms of a three-man crew, consisting of a Soviet commander and a flight engineer and research engineer from other Comecon countries. This suggests either a return to re-entry in shirt-sleeves or else the use of a larger spacecraft, and Shatalov has indicated that "in time" larger orbiting stations will be used, making it possible for the crews to include specialists from different fields. But he did not make it clear whether such stations would be in use by 1983, and present plans for Comecon participation go no further than this date.

It remains to be seen whether such joint missions form an integral feature of long term Soviet space planning, or whether, as in the case of the flight of the first (and only) woman cosmonaut Valentina Tereshkova, a policy hailed as a triumph of socialism may later be quietly phased out. The manned programme, however, is by no means the only new development of Interkosmos, nor in the long term is it the most significant.

Petrov and Gröte speak rather

vaguely of Comecon participation in "still more complex and interesting experiments". This may mean no more than the provision of equipment for Soviet spacecraft, a development that is virtually implicit in the "integration" policy which aims at increasing specialisation of the member countries. But Comecon is also to provide some of the ground facilities. Already the Comecon countries track the Soviet spacecraft. The accession of Cuba to Comecon provided an important new link in the network; new plans include the provision of various research facilities. A new astronomical observatory was recently opened in Prague as part of the Interkosmos programme.

Poland's contribution will be considerable—a Space Studies Centre within the Polish Academy of Sciences to include institutes of space physics and geodesy, earth and environmental resources, the application of space technologies, materials testing, and also a computer centre. According to Professor Stefan Piotrowski, a member of the Polish Academy of Sciences and also of the Interkosmos Council, the centre will work on methods of research into the effect of solar

activity on the ionosphere and atmosphere which, he says, will benefit communications, meteorology, agriculture and possibly medicine. The council will also draw up a programme of experiments to be carried out by manned spacecraft and satellites.

Although such facilities are designated part of the Interkosmos programme, it seems inevitable that the Soviet Union's own national space programme will also benefit from access to them; indeed, this would provide a further opportunity for "fraternal cooperation". However, certain joint Comecon projects have already evoked the criticism that the Soviet Union has made little contribution other than a site or the expertise, while the financial burden has fallen on her partners. However great the official acclaim of such cooperation, the possibility remains that those scientists already disenchanted with Comecon countries may view the new developments in the space programme not so much as a triumph for fraternal integration as a way for the Soviet Union to get routine research and development done at someone else's expense. □

NETHERLANDS

An unfavourable climate

Casper Schuurings reports on recent developments in science policy in Holland

THE Dutch Government is going to spend 2,560 million guilders (about £640 million) for research in 1977. Universities will use less than half (1,130 million), after a budget this year of 2,300 million. Industry plans to have 2,810 million guilders available next year for research and development after 2,510 million this year; at the end of last year industry had expected to spend 2,190 million in 1976, indicating that the climate for investment in research is not as favourable as it seemed. Some 390 guilders will be spent on research and development per head of the population.

These figures are part of the new budget presented to parliament by the science policy minister, Fokele Trip. He says science policy cannot be seen apart from the socio-economic development of the country: out of science policy a contribution should come to the selective growth of the economy which the government has outlined in the government memorandum on industrial policy issued earlier this year. Environmental factors, town and country plan-

ning and a policy to avoid the wasteful use of energy and resources all form part of that selective growth policy.

On the energy front 60 million guilders will be available for research as the specially formed fund for the sodium-cooled fast breeder reactor in Kalkar will be abolished. This will be used mainly for programmes other than in the nuclear field. It is surprising, however, that in the plans for research up to 1981 which were presented along with the science budget, the nuclear energy component will increase while the total energy research and development budget will hardly be more in 1981 than in 1977. The future for alternative energy sources is therefore looking very bright at the moment.

Mr Trip is also planning to spend more money for a popularisation of science. For five years the University of Amsterdam has organised courses for scientists to write articles for a larger audience beyond their own discipline, and it will now receive a government subsidy. Other universities are also starting such a course. At the School for Journalism a training-course for science writing will also be introduced with ministerial money, and the Royal Dutch Academy of Sciences is conducting a study on a science information unit to improve "science-



Dutch Embassy

Trip: not available

society" communications; Dutch universities and some research institutes are, however, already more or less active in this respect.

The science budget received a favourable reception in the parliament's special commission on science policy. Mr Trip has said that for personal reasons he is not available for a government post after the elections in May 1977, and feels that a new minister for science policy is necessary. He says he will leave behind a sort of "last will" describing how best the position of such a minister can be used. □

IN BRIEF

Nuclear trade

Export of Australian uranium ore to the United States, Japan and West Germany, embargoed for four years, is to be increased but not matched by an extension of mining operations. Export permits are to be issued for Rio Tinto-Zinc to meet existing contracts, but exploitation of other mines must await the further findings of the Fox environmental enquiry.

Canadian sternness over the proposed French sale of a nuclear reprocessing plant to Pakistan has meanwhile resulted in France letting it be known that if Pakistan chose to cancel the contract no strong objections would be raised. The French have no intention of backing out themselves, in spite of modifications in their nuclear export policy acknowledging the dangers of nuclear proliferation. Canada provided Pakistan's only nuclear reactor, and has threatened to supply fuel for only

two years instead of six if Pakistan acquires a reprocessing plant.

● The informal association of nuclear exporters which met in London last week has grown to 15 with the addition of Switzerland, with observer status only. Members of the group have now been named officially. They are the United States, the Soviet Union, France, Britain, West Germany, Japan, Canada, Belgium, Sweden, The Netherlands, Italy, Switzerland, East Germany, Poland and Czechoslovakia.

Genetic proposals: reactions

The Institute of Biology has now expressed its concern about the UK Health and Safety Commission's proposals for regulating genetic manipulation experiments in Britain. Commenting last week on the Commission's consultative document, which was circulated at the same time as the Williams Committee report, the Insti-

tute says it is "neither practicable nor desirable" to have both a blanket regulation requiring advance notice to be given on all experiments and a clause exempting "what is likely to be over 90% of microbiological experiments".

The Commission, the Institute says, "has not available, nor will it be able to recruit, sufficient suitably qualified staff to deal with notifications and exemptions", and goes on to suggest that its involvement should be "solely as a body able to enforce recommendations" of GMAG, the advisory group recommended by Williams and now being established by the DES.

The Association of University Teachers (AUT) also made its views known last week. Making similar criticisms to those of the Institute of Biology, the AUT asks that the Commission think again "on the lines that it puts forward a much stricter definition of the experiments covered".

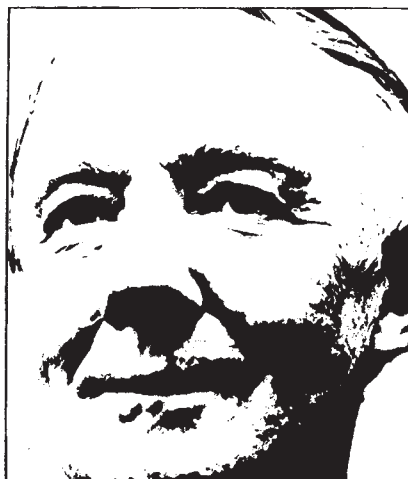
OUR farm animals, as they exist today, have been produced by selective breeding from wild ancestors. Domestic cattle in Europe and North America grow rapidly when kept warm and dry with an ample supply of nutritious feed, and they give milk in large volumes under similar, unnatural conditions. Unless grazed on lush pastures of cultivated grasses whose productivity is maintained by the plentiful use of fertilisers, or unless they receive concentrated high-protein rations, their yield of meat and milk falls well below their potential. So long as the world produces a surplus of food to support this type of husbandry, the breeds which are at present the most numerous—the Friesian and the Charolais for instance—will retain their popularity.

As new and more productive types of cattle have been produced, so many of the older breeds have become less common, and some are already extinct. Similar processes have operated with sheep, pigs and poultry. The variety of the livestock on our farms is rapidly being reduced.

Most farmers have welcomed the simplification of having only a few well-characterised breeds of animals to choose from, and have not been worried by the disappearance of so many others. However, there has recently been more concern about the loss of so many breeds which have evolved during the last two thousand years, particularly as the original ancestors of the breeds cannot now be identified. Organisations such as the Rare Breeds Survival Trust have been established, and the results of

their efforts can be seen at the Cotswold Farm Park in England, and in Folk Museums in Scandinavia.

Many who wish to save our old breeds probably do so for senti-

Past breeder**KENNETH MELLANBY**

mental reasons, but they also justify their policy by suggesting that it has an educational value, to teach children and students about the history of farming. It is also claimed that some of the old breeds may be able to contribute valuable genes to future breeding programmes, and thus produce animals with virtues missing from existing commercial strains. Plant breeders have used ancestral forms of potatoes and cereals in this way, and animals might be treated in a similar manner. Unfortunately, though this may still be a possibility, most

breeders of cattle and sheep think that there is little valuable genetic material in the small surviving stocks of most old breeds. This opinion may be coloured by their preoccupation with animals suitable for intensive farming, where hardiness and the ability to survive are no longer sought.

I believe that we should look again at some old breeds as meat producers in their own right. As the world population grows, there will be less surplus food for intensive animal rearing. Our best land, in all countries, will be increasingly needed for crops for human consumption, and marginal land in the uplands will become more important for raising livestock. Already it has been shown that non-domestic species such as the Red Deer in Scotland and the Eland in Africa may produce meat more efficiently than cattle or sheep on poor grazing, and some scientists think we should make more effort to find new species to domesticate. Unfortunately deer and antelope have proved difficult to manage in large numbers, so their potential is limited.

Some of the old breeds may be as good at making meat from poor herbage, and we may benefit from their heritage of domestication. I have kept Soay sheep, the breed still found on the islands of St Kilda off the west coast of Scotland and apparently little changed since they were herded by Neolithic man, on pasture on which ordinary domestic breeds would have starved. The animals flourished and bred. Other breeds of sheep and cattle, unsuitable for the intensive farm, might do equally well.

correspondence

The toxicity of plutonium

SIR,—In your editorial "Take your time Mr Benn" published on September 30 you discuss the Report of the Royal Commission on Environmental Pollution. You state that "the report first reviews what is known in radiobiology with particular attention to plutonium", and that "independent consultants were brought in. . . . The consultants' reports have not been published." Two of the signatories of this letter (F.W.S. and J.V.) are acknowledged by name in paragraph 537 of the report. We wish to make it clear that the two other signatories (R.D. and P.L.), who are members of the Commission, take full responsibility for Chapter II on Radioactivity and Radiobiology in the report. This was not seen by the consultants at any time prior to publication. In fact they disagree with several of the statements made in this chapter.

In paragraph 66, for instance, the statement that "plutonium isotopes are retained in the body once they gain admittance" is certainly misleading. Excretion does occur though it is not rapid, while no mention is made in paragraph 55 of the salient fact that the energy deposited in the tissues by the strontium 90 decay process is about 200 times greater than that deposited by tritium.

Further, the greater part of the evidence given to the Commission by the consultants has already been published in their joint paper in *Nature* (February 19, 1976) entitled "Hazards of plutonium with special reference to the skeleton", and in a paper by Janet Vaughan entitled "Plutonium—a possible leukaemic risk", in W. S. S. Jee (Ed.), *Health Effects of Plutonium and Radium*, 691–705, (J. W. Press, Salt Lake City, Utah; 1976). In fact references to these two papers were made by the Commission in Chapter II and in their bibliography.

In their written evidence to the Commission the consultants stated "We are in agreement with the conclusions arrived at by the Medical Research Council¹ and the National Radiological Protection Board² on the risk to the lung and on the question of the hot particle." Reference to both these matters was made in their paper published in February in *Nature*, and in a paper published by Mayneord and Clarke³ on which their discussion was

partly based and which was also published in the same issue of *Nature* under the title "Quantitative assessment of carcinogenic risks associated with hot particles". The substance of the consultants' comments on papers submitted by the National Radiological Protection Board on the numbers of deaths from leukaemia at the Windscale reprocessing plant are contained in paragraphs 74 and 75 of the Report.

Yours faithfully,

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¹ *The Toxicity of Plutonium*, Medical Research Council, HMSO (1975).

² Dolphin, G. W., Smith, H., Popplewell, D. S., Stather, J. W., Adams, N., Spoor, N. L., Brightwell, J., and Bulman, R. A., *Radiological Problems in the Protection of Persons exposed to Plutonium*, NRPB R.29 (1974).

³ Mayneord, W. V., and Clarke, R. H., *Nature*, **259**, 535 (1976).

Genetic manipulation

SIR,—The contribution of John Locke, the Director of the Health and Safety Executive, to the debate on genetic manipulation raises a new and disturbing issue. The Director claims that "the techniques described as 'genetic engineering' should be permitted where they offer prospects of social benefit" subject to safety precautions. So we learn that the safety of experiments is not the only criterion on which they are to be judged; officials are also to pass judgment on the social benefit of genetic experiments.

The ethical problems were not even discussed in the Williams Report, which confined itself solely to safety considerations in genetic manipulation. Clearly the Health and Safety people have no mandate to assess the social benefits of work. Besides the safety aspects of work in genetics, ethical problems could arise and these need very full debate before they have any in-

fluence on executive action.

Yours faithfully,

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Human anatomy

SIR,—The interest in descriptive asymmetry shown in *Nature's* pages indicates that the classic German treatise on the subject¹, reprinted in 1970, deserves recognition by the English-speaking world. Asymmetry of human limbs, and much else, has a large literature but lacks a recent review. There is even a more recent paper² devoted to a positive correlation between handedness and scrotal asymmetry. There are also by now several dozen analytical treatments^{3–5}; fluctuating asymmetry (but not other kinds) is a measure of the imprecision of development.

Yours faithfully,

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¹ Ludwig, W., *Das Rechts-Links Problem im Tierreich und beim Menschen* (Berlin, Springer; 1932).

² Chang, K. S. F., Hsu, F. K., Chan, S. T., and Chan, U. B., *J. Anat.*, **94**, 543–548; 1960.

³ Van Valen, L., *Evolution*, **16**, 125–142; 1960.

⁴ Whitten, M., *Genetics*, **54**, 465–483; 1966.

⁵ Bailit, H. L., Workman, P. L., Niswander, J. D., and Maclean, C. J., *Human Biol.*, **42**, 626–638; 1970.

⁶ Jackson, J. F., *Syst. Zool.*, **22**, 166–170; 1973.

⁷ Salzano, F. M., and Benevides, F. R., *Amer. J. Phys. Anth.*, **40**, 325–328; 1974.

⁸ Siegel, M. I., and Doyle, W. J., *J. Exper. Zool.*, **191**, 211–214; 1975.

Careers in science

SIR,—There has been a great deal of discussion recently about possible short term cutbacks in 'big science' projects. Nowhere have the effects on the careers of scores of research students been discussed. Simultaneously there are complaints about the lack of prospective students for university science courses. Is it not possible that these two sets of circumstances are directly related?

(Name and address supplied)

To be or not to be

SIR,—Isn't isn't isn't! (October 7, page i).

Yours faithfully,

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news and views

Coexistence with insect pests

from Robert M. May

INSECTS have shared man's crops, and transmitted his diseases, from the earliest times. On a tomb erected towards the end of the Old Kingdom in Ancient Egypt (about 2,300 BC) locusts are shown eating cereals, and the Book of Joel (I, verse 4) records a remarkable sequence of entomological misfortunes wherein "that which the palmerworm hath left hath the locust eaten; and that which the locust hath left hath the cankerworm eaten; and that which the cankerworm hath left hath the caterpillar eaten". Today at least one person in six is suffering from some form of insect-borne disease (primarily 300 million from filariasis and 200 million from malaria; see *Nature*, **262**, 85; 1976), and world crop losses are estimated around 10–15%.

Agricultural societies have evolved a variety of empirical practices aimed at reducing the depredations of crop pests. In the absence of detailed understanding of the pest's population dynamics, it is hard to be sure whether any one such practice represents the optimal control strategy, or the best among similar strategies (but inferior to some qualitatively different strategy), or simply ignorance hardened into habit. Chiang (*Science*, **192**, 675; 1976) has reviewed a most interesting series of Chinese texts, which *inter alia* codify this sort of folk wisdom: it is advised, for example, that the top leaves and buds of cotton plants should be examined at 3-day intervals for eggs of the cotton bollworm, and chemical control initiated above a threshold of 15 eggs for 100 plants.

An analytical approach to the control of crop pests and forest defoliators has been pioneered by Watt, Holling, Huffaker and others. Here one starts with a fairly detailed model for the population dynamics, and tries to determine the relevant parameters from field and laboratory experiments: in effect one tries to decide whether 5 or 15 or 50 is the appropriate number of bollworm eggs, or whether some qualitatively different strategy may be better. A notable example of this approach centres on the froghopper, *Aeneolamia varia saccharina*, which

attacks sugar cane in Trinidad and elsewhere (Conway *et al.*, in *Study of Agricultural Systems*, edit. by G. E. Dalton; Applied Science, London, 1975; and Conway, chapter 14 in *Theoretical Ecology: Principles and Applications*, edit. by May, R. M.; Blackwell, Oxford, 1976). Starting from a basic model for the dynamical behaviour of the population, the effects of various control strategies are determined. The economic costs and benefits of these strategies are then calculated, in Trinidadian dollars. The optimum strategy turns out to depend mainly on the size of the first-generation brood of hoppers, and thence on the weather. Optimal control can nevertheless be maintained by use of dynamic programming techniques.

On a more general level, Southwood (in his opening address to the 15th International Congress on Entomology, in Washington, DC on August 18) and Conway (*op. cit.*) have recently used contemporary ecological notions to bring some order and pattern to the bewildering variety of dynamical behaviour of insect pests.

At one end of a continuum are the 'r pests' (*Nature*, **257**, 737; 1975), which are characteristic of unpredictable, transient or patchy habitats: examples are desert locusts or fruit flies. Their strategy is one of boom and bust, and r pests will virtually always achieve pest level if enough of them invade the crops and there is adequate time before harvest. At the other extreme, 'K pests' occur in stable habitats. Their populations are typically steady and relatively low, and they do not have a serious impact on their natural food. K pests achieve pest status when their low level of harvesting is nonetheless unacceptable to man (as is codlin infestation in a fraction of the apple crop, or ectoparasite holes in a commercial hide), or when the introduction of plants or animals to new areas disturbs natural balances (the mirids that, even at low population levels, are destructive of the cocoa trees introduced into West Africa and the Far East inflict relatively little damage on their natural host plants;

trypanosomiasis, carried by tsetse flies, is rarely lethal to the indigenous African fauna, but is a problem for man and his domestic animals). In between these extremes are the majority of deciduous forest defoliators, fruit insects and some vegetable pests. These 'intermediate pests' are for much of the time held at a lower equilibrium point by natural enemies, but they occasionally escape this natural control and reach outbreak levels. Those near the r end of the spectrum, such as aphids, escape most frequently. Intermediate pests may become r pests when they are introduced to new areas where their natural enemies are absent: examples of such introductions, where the organisms are not significant nuisances in their countries of origin, are the gypsy moth, *Lymantria dispar*, to North America, and the giant African snail, *Achatina fulica*, to Pacific islands. This continuum of r, intermediate and K pests is elaborated and fleshed out with numerical examples in Southwood and Comins (*J. Anim. Ecol.*, **45**, 949; 1976).

Southwood observed in his opening address that "this knowledge of the underlying population dynamics of different pests gives useful indications of the appropriate pest control methods". The term control is here used in a precise and sensible way to mean reducing the pest impact to a level where the marginal cost of further measures would exceed the marginal revenue to be gained thereby.

In Southwood's words, "r pests are always fluctuating, and therefore any appeal to the stability of natural ecosystems is spurious and foredoomed to failure". Cultural control techniques will depend on reducing the chances of pest invasion. Paradoxically, and contrary to tenets laid down in simple ecology tracts, one way of doing this is to increase the scale of a monoculture, thus increasing the area-to-perimeter ratio and heightening the degree of isolation (Way, in *Biology in Pest and Disease Control*, edit. by Price-Jones and Solomon; Blackwell, Oxford, 1974). However, in these "inherently booming populations, in-

secticides will remain the most powerful technique: their rational use will demand the development of better methods of forecasting and assessing pest outbreaks".

Intermediate pests should be kept below their release point, so that the population is stabilised by natural enemies. Southwood emphasises that these are the pests against which biological control, or integrated control with a significant natural enemy component, must be the dominant strategy. When pests of this intermediate kind are present in an ecosystem, the mindless "prophylactic" application of insecticides is likely to eliminate the lower equilibrium point controlled by natural enemies, thus leading to outbreaks of "secondary" or "upset" pests.

K pests will be the most susceptible to eradication, or at least to being kept at low population levels. They often have complex reproductive tactics and these, together with the relatively low rate of recruitment of the adult stage, make them vulnerable to techniques such as the release of sterile males (as

in Knipling's work on the screw-worm fly, *Cochliomyia hominivorax*) or the use of pheromones.

These insights imply that insecticides are likely to remain a vital tool in engineering our coexistence with insects. On the other hand, the past 30 years have seen a faster-than-exponential rise in the proportion of pests resistant to insecticides: over 200 species of significant pests are now resistant to one or more chemical compounds. Eradication programmes based on the heavy, widespread and prolonged use of insecticides are not only biologically naive and usually futile, but they also squander a valuable resource—the life of the insecticide. The obvious conclusion, emphasised by Southwood and by the US National Academy of Sciences study committee on pest control (*Science*, 191, 836; 1976), is that we must in the future be more selective in our use of insecticides, and more efficient in their application, than we have been in the past. In this endeavour, analytical understanding of the population dynamics is a crucial ingredient. □

Giordano Bruno, the Moon's latest large crater

from David W. Hughes

To the observer with a telescope the Moon's surface seems unchangeable and over three centuries of study has revealed no well documented alteration. This is hardly surprising considering that, in the absence of erosion, the cratered surface has taken more than four thousand million years to get to its present state. Also recent estimates of the present day cratering rate (Shoemaker, *NASA Tech. Rep. No. 32-700*) indicate that a 1-km crater (which is just on the seeing limit for Earth-based telescopes) will only be formed on average once every 4×10^7 yr. A crater of 20 km or larger is produced every 3×10^{11} yr, which in view of the fact that there are many such craters on the Moon is ample indication of how the large body influx and thus the cratering rate has decreased with time.

The above points make the paper by Jack Hartung (State University of New York, Stony Brook) in the recent edition of *Meteoritics* (11, 187; 1976) all the more fascinating. Hartung retells the report of five men who happened to be looking at the new Moon early in the evening of July 18, 1178. The Moon was then 1.5 d past new and would have been seen as a thin waxing crescent setting near the western horizon in the evening twilight glow.

"... suddenly the upper horn split in two. From the midpoint of this division a flaming torch sprang up, spewing out over a considerable distance fire, hot coals and sparks. Meanwhile the body of the Moon which was below writhed, as it were in anxiety ... and throbbed like a wounded snake. Afterwards it resumed its proper state. This phenomenon was repeated a dozen times or more, the flames assuming various twisting shapes at random and then returning to normal. Then after these transformations the Moon from horn to horn, that is along its whole length took on a blackish appearance".

This report was given on oath to Gervase of Canterbury and incorporated in his mediaeval chronicles (edit. by Stubbs, W., *The Historical Works of Gervase of Canterbury*, 1, Her Majesty's Stationery Office, London, 1879).

It is highly unlikely that some Earth-based event caused this phenomenon although an unusual cloud layer or turbulence in our atmosphere or the entry of a meteoroid along the line of sight to the Moon could have been responsible. The report, however, repeatedly refers to the Moon and does not mention clouds or sky specifically.

Hartung suggests that the description

is consistent with the occurrence of a large impact on the lunar surface. "The upper horn split in two"—part of the sunlit crescent became obscured by the cloud of ejecta produced by the impact. "A flaming torch sprang up, spewing out fire, hot coals and sparks"—a vivid description of the hot dust, molten rocks and incandescent gases ejected from the impact point. "The moon writhed and throbbed like a wounded snake"—the Moon seen through the highly turbulent temporary atmosphere produced by the ejecta. "This phenomenon was repeated a dozen times or more"—the production of secondary impact craters by large pieces of ejecta that had insufficient energy to escape from the gravitational attraction of the Moon and fell back onto its surface. "The Moon then took on a blackish appearance"—the entire Moon's now having a temporary dusty atmosphere which blocked a significant amount of the light reflected from its surface.

Does such a newly formed crater exist on the Moon? The event occurred near the midpoint of the upper horn of the new Moon so the area around latitude 45° north, longitude 90° east is a good place to start looking. It was also easily visible to the naked eye indicating that the ejecta cloud was more than 100 km across. The crater should therefore be more than 10 km in size. Also a recent crater should have a bright ray system (rays being constant-width fingers of light coloured ejecta which radiate from the area of the central crater along the arcs of lunar great circles).

Searching the area between latitude 30° and 60° N and longitude 75° and 105° E using Lunar Orbiter and Apollo mission photographs Hartung soon found a likely candidate, the crater Giordano Bruno (latitude 30° N, longitude 103° E). This is 20 km in diameter and is surrounded by very prominent bright rays which extend for hundreds of km (see NASA photograph AS8-12-2209). In fact Giordano Bruno has the largest ray length-to-crater diameter ratio of all the lunar craters. The crater rim is also very sharp and has not yet been rounded off by the "gardening" process as have the older craters (Fig. 1).

It is interesting to think how this theory can be verified. Assuming that the observation was made near Canterbury, Hartung finds that on June 18, 1178, the Sun set at about 20.15, the Moon setting about 45 min later, so the event must have occurred between 20.15 and 21.00 GMT. Also making the assumption that the Sun had to have set for the event to be visible, limits the points on the Earth from which it could be seen to a band stretching from the region between

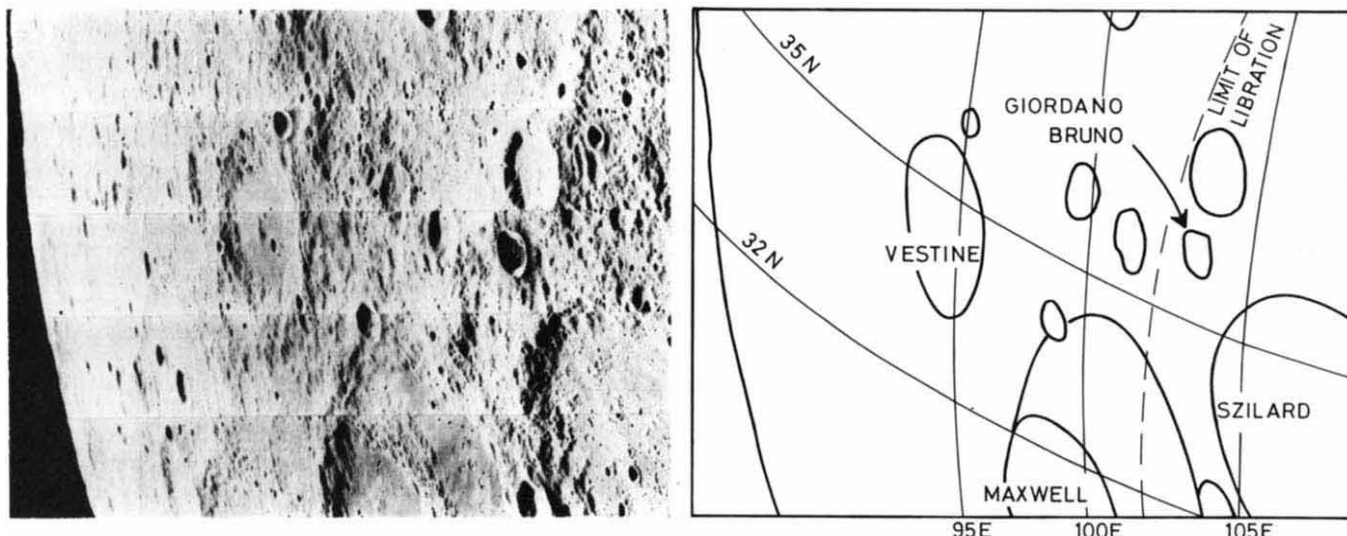


Fig. 1 A Lunar Orbiter photograph of the Moon's latest large crater, Giordano Bruno. The impact producing this crater was observed from near Canterbury, England at around 20.40 Greenwich Mean Time on June 18, AD 1178 (courtesy of NASA).

Oslo, Norway and Stockholm, Sweden, through southern England and the north-west corners of France and Spain, west of the Canary Islands and along the south-east coast of Brazil. Mediaeval records from these countries should be searched to see if the event is mentioned.

The crater itself might still have the signature of its recent formation. Microwave sensors on board a lunar polar orbiter may still be able to detect a higher temperature inside the crater, attributable to the heat generated during the original impact. Also rocks ejected from the crater will have been exposed to space for only 800 yr and will have a distinctive population of micrometeorite craters and cosmic ray particle tracks. The return of such samples to Earth will not only prove the craters to be of recent origin but will also be a valuable clue to other long time-scale Solar System processes such as the implantation rate of solar wind particles and cosmic ray nuclear reaction rates.

All in all this crater identification is most exciting. Even taking Baldwin's (*Icarus*, 14, 36; 1971) Earth cratering rate as applicable to the Moon the chances of anyone observing the formation of a large lunar crater on the Earth-facing side of the Moon during the millennia of recorded history (say 3,000 yr) is 1 in 2,000. Because observations have not been made continuously, may not have been recorded or the records might have been lost, the probability becomes extremely small and the Canterbury record even more fortuitous. Discounting the dubious and controversial changes claimed to have occurred to the crater Linné in Mare Serenitatis (Fauth, *Astr. Rundschau*, 3, 172; 1901) Giordano Bruno is unique, being the only large lunar

crater to be formed in recent history and as such it becomes a prime lunar target for future space exploration.

Drugs and body temperature

from a Correspondent

The Third Symposium of The Pharmacology of Thermoregulation, was held at Banff, Canada, on September 14–17, 1976.

"AND now for something completely different!" This sentiment imbued many of the presentations at the conference. 'Thermopharmacology', a term coined in 1969, is devoted to the relation between the action of a drug and an animal's body temperature in all its complexity, and it is difficult to know where pharmacology ends and physiology begins in this subdiscipline. In fact, much of this conference did deal with functional rather than pharmacological questions—particularly those centred on the body temperature control mechanism located in the brain. Overall, there were several exciting highlights.

"What is the purpose of a fever and what is its value to the survival of the organism?" In asking this question, M. Kluger (University of Michigan Medical School) laid a potential bombshell in the physician's lap. In an elegant experiment, he demonstrated that aspirin is not beneficial after all

when given to a rabbit, desert iguana or other animal during a fever caused by bacteria. In fact, if given a dose of aspirin that prevents fever from developing, a large proportion of the animals die. Following on, J. Covert and W. Reynolds (Pennsylvania State University) reported independently that goldfish, small-mouthed bass and other fish manifest a "behavioural fever". After infection with bacteria, the poikilotherm, living in an aquarium heated along a gradient, swims to warmer water. If kept in cooler water, the bacteria-laden fish dies. If an aspirin-like compound is dissolved in the aquarium water, an afflicted fish will likewise succumb, since it fails to swim to warmer water after the antipyretic drug is taken in, presumably through the gill network. Even though a fish does not regulate its body temperature in the same way as a mammal, the nervous system of this evolutionarily more primitive animal is nevertheless affected by the pathogen. So aspirin usage may have been dealt yet another blow.

A new "activator substance" released by white blood cells was described by G. Gander (Medical College of Virginia). This protein possesses the property of triggering other types of inflammatory cells to release a pyrogen. The "activator" could account for the fever seen in a patient who is stricken with a heart attack yet exhibits no signs of an infectious agent. Observations by E. Atkins (Yale University) implicate a lymphokine released from blood lymphocytes that causes other blood cells (monocytes) to produce pyrogen. A fascinating question now is whether the lymphokine and the "activator" are one and the same.

Along these lines, a new assay for

identifying "pyrogenicity" (the fever-producing capacity of a substance) was evaluated by G. Meers (Calgary Medical School). If *Limulus* (horseshoe crab) blood is mixed in solution with quite minute amounts of bacteria, the crab's blood thickens. Conceivably, in a patient who has contracted an infectious disease, bacterial counts in blood could be quantitated; alternatively, the crab blood assay could be used for the relatively rapid screening of drug solutions intended for administration to the human patient.

In several quarters, pockets of distinct but congenial controversy arose. What, for example, is the role of the metabolic substrate cyclic AMP in the temperature control mechanism? A. Milton (University of Aberdeen) reported that cyclic AMP and other cyclic nucleotides produce hypothermia on their injection into the hypothalamus of the cat. But exposure of the animal to a hot or cold environment fails to alter the central level of cyclic AMP. A contrary result was then presented by C. Rosendorff (University of Witwatersrand, Johannesburg) who contended that, in the rabbit, cyclic AMP evokes hyperthermia and indicated that cyclic AMP could be an intermediary for producing fever. An easy resolution would be to fall back upon "species difference" as an explanation. Recent evidence continues to accumulate which implies that different mammalian species have similar neural and humoral mechanisms for heat gain and heat loss. In fact, it was revealed that in the rabbit 5-hydroxytryptamine (5-HT) causes hyperthermia, and noradrenaline, hypothermia, which are precisely the same responses seen in the cat, rat, monkey and other species. Expertise in technique, or lack thereof, rather than nature's capriciousness may ultimately explain the so-called "species difference". In any event, the "second messenger" question concerning cyclic AMP has yet to be resolved.

An even more intriguing controversy is the role of the prostaglandins (PG) in the brain and the part they play in fever induced by bacteria. Ostensibly, bacteria cause an increased synthesis and/or release of a PG in the brain which subsequently produces a long-lasting hyperthermia. R. Hellon (National Institute for Medical Research, London) shook the theory by announcing that an antagonist of PGE₂ (a compound coded as SC 19-220) blocks a fever evoked by this prostaglandin. Thus, if the pyrogen-PGE theory were correct, this antagonist should likewise prevent bacterial fever. But it does not. This suggests an alternative mediator. R. Myers (Purdue University) troubled

the waters further by showing that a low dose of aspirin perfused in the cat's hypothalamus, although blocking PGE activity in this structure, fails to prevent a bacterial fever from developing. However, 5-HT activity in the hypothalamus increases simultaneously with the rise in body temperature after infection with bacteria. Thus, 5-HT may be the mysterious intermediary in the hypothalamus after all. C. Rosendorff (Johannesburg) informally countered that a part of the PGE hypothesis may still be valid, since a precursor of PGE or a factor such as an endoperoxide (thromboxane) in an alternative pathway could be causal intermediaries. Feldberg ended by describing how PG-induced and other fevers can now be examined in the anaesthetised animal, given the appropriate anaesthetic. The brain mechanism responsible for fever and its development is still tantalisingly elusive.

Ionic mechanisms in the hypothalamus also exert an apparent influence in the final common neuronal pathway underlying temperature control. D. Jones (Calgary Medical School) showed that the hypothermia induced by calcium ion perfusion of the cat's hypothalamus is antagonised by adding glucose to the perfusate. This observation suggests that a local energy substrate can modify the ionic activity of the posterior hypothalamus. The endogenous activity of Ca²⁺ ions, traced in the same region of the cat's brain by R. Myers (Purdue), changes in an opposite direction when the animal is warm or cold as well as when its hypothalamic thermoreceptors are warmed or cooled. In spite of this, the issue of whether or not an ionic mechanism does underlie the typical set-point temperature of 37 °C persists.

Ingenious techniques and their use were also emphasised. For example, the behavioural assessment of drugs that affect temperature regulation is now a popular procedure for deciding whether or not an animal will try to avoid being cold or warm after a drug is administered (E. Adair, Yale; E. Satinoff, University of Illinois). Although the importance of diurnal rhythms was also forcefully stressed by F. Halberg (University of Minnesota), one wonders whether the use of a control animal at the same time of day or night as an experimental subject would really make it possible to interpret let us say, a thermal effect of a given drug. Finally, the radioactive microsphere techniques developed recently were described by R. Hales (CSIRO, Sydney). These techniques are useful in obtaining a comprehensive picture of the effects of thermal stimulation on the regional distribution of cardiac

output and blood flow.

With the advent of the Feldberg-Myers monoamine theory of thermoregulation more than 10 years ago, pharmacologists have often concluded that a drug affecting body temperature does so by altering the balance between 5-HT and noradrenaline activity in the animal's hypothalamus—the essence of this theory. At the conference, it was evident that endogenous substances other than these amines will have to receive equal time at the research bench. Thyroid releasing hormone (TRH), prostaglandins, calcium, dopamine and other substances could be important factors mediating the brain's response to a drug given systemically. Of course, the relevance of these substances to synaptic events with regard to the hitherto prominent role of biogenic amines is still uncertain. □

Control systems in higher plants

from John A. Milburn

A symposium entitled Integration of Activity in the Higher Plant was held in Durham, under the auspices of the Society for Experimental Biology on September 4-9, 1976. The proceedings will be published.

BUMBLE-BEE flight physiology and rocket guidance systems are unexpected topics to meet in a plant physiology symposium. Nevertheless such examples served to illustrate the theme of the meeting, although those expecting papers on integration may have been disappointed; most emphasis was on control systems, probably because information on integration is so sparse.

The regulation of the heating up of the *Arum* spadix has been studied by T. ap Rees (University of Cambridge). Biochemically, the *Arum* spadix is a curiosity: in nature it heats up to more than 10 °C above ambient temperature, releasing a scent that attracts *Psychoda* midges which pollinate the flower. Heating is achieved by intense respiration comparable to that in the most physiologically active tissues such as the flight muscle of bats, birds and insects. This exaggerated respiratory system is used by ap Rees to study the inter-relation between glycolysis and the pentose phosphate pathways. A search for a mechanism in plants like the 'free-wheeling' or 'futile' cycles, responsible for warming up humble-bee flight muscles in cold weather, has not been successful in *Arum*.

Why do stored seeds lose their viability? D. J. Osborne and colleagues (ARC Unit of Developmental Botany, Cambridge) attribute the loss of capacity of rye grains to germinate, to the progressive proliferation of embryonic lesions. With time, lesions in embryonic tissues increase beyond the point at which germination is possible, producing a state in which rye is 'deader than dead'. Lesion formation seems to result in failure to reactivate nucleic acid and protein synthesis sufficiently rapidly. Curiously, if rye grains are subjected to a preliminary brief wetting and drying cycle before sowing, reactivation is improved and viability and embryo vigour are enhanced over controls.

One would think that the flow of the transpiration stream through plant organs would have few mysteries by now since the water flow is generally believed to be regulated predominantly by the physical properties of the cell walls. On this basis the hydraulic conductance should be constant irrespective of the water potential gradient. J. S. Boyer's (University of Illinois, Urbana), results, like those from other laboratories, show that conductance increases dramatically as the water pressure gradient increases, which is not expected of a simple physical system. There was no obvious agreement as to the explanation of this intriguing phenomenon.

Generations of students have deduced cell turgor pressures by immersing pieces of tissue in osmotic solutions on the assumption that the internal solute concentration remains constant. This assumption is now strongly challenged by U. Zimmermann (Kernforschungsanlage, Jülich) who has measured the turgor pressure of single cells using a microprobe and pressure transducer. It is clear that cells respond directly to turgor pressure because, in addition to the anticipated water transport, the injection of inert fluid into cells produces immediate changes in the fluxes of solutes, especially potassium. Similar methods have been used to determine the elastic modulus of *Valonia* and *Mesembryanthemum* cells. How pressure modifies ion fluxes is a mystery. The geometry of the plasma membrane is apparently disturbed but the full explanation will be more complex. Although cell membranes can be compressed mechanically the technique is tedious but Zimmermann has introduced a revolutionary electrical method which charges membranes in the same way as the dielectric of a capacitor. Many new advances can be expected of this technique which has already been used to punch temporary holes in erythrocyte mem-

branes, causing them to absorb drugs. These erythrocytes can be reinjected and are being tested for greater control in the treatment of cancer.

The regulation of ions and solutes in cells and plant organs was outlined by J. A. Raven (University of Dundee), then M. G. Pitman and W. J. Cram (University of Sydney). Nowadays the driving system supplying energy for ion transport is widely believed to depend on proton pumping in accordance with Mitchell's chemiosmotic hypothesis. Proton pumping, if linked directly or indirectly to other ionic fluxes can explain many physiological responses, such as stomatal regulation, auxin transport and cell expansion, in addition to ion uptake by plant roots. The lipid bilayer of cell membranes has a high electrical and hydraulic resistance and to drive solutes at the rates commonly observed the membranes must contain ion pumps. The regulatory control of ion pumping is seen in the response to specific ions and also to growth regulating substances. In whole plants the uptake of cations and anions tends to be balanced and proportional to the amount of shoot growth but how the plant maintains the correct balance is not known. Is carboxylic acid production in leaves sufficient to balance cation uptake, for example, and is the extrusion of hydrogen ions from roots sufficient to explain cation uptake?

The orientation of plant organs requires sensing and response systems analogous to those in guided rockets, according to M. B. Wilkins (University of Glasgow) explaining recent progress in research on phototropic and geotropic responses. A strong plea was made for greater precision in describing and interpreting "hormone" experiments. Bioassays are too ambiguous for critical work and must be supplanted by physicochemical techniques such as mass spectrometry, used recently to confirm that the coleoptile geo and phototropic responses are in fact caused by indole acetic acid (IAA). Control systems are complex, varying between different organs and tissues and involving an interplay of growth promoters and inhibitors which operate in minute dosages. In *Zea* roots, growth inhibitors are undoubtedly involved in geotropic curvature, current evidence favouring a role for abscisic acid (ABA) rather than IAA. Doubt was cast on the popular view that sedimenting starch grains initiate the geotropic response.

Two theoretical papers by I. R. Cowan (Australian National University) and J. H. M. Thornley (National Vegetable Research Station, Littlehampton) gave refreshingly clear ex-

positions of mathematical model building. Their models sought to explain how variations in stomatal aperture might optimise productivity in arid zones and how crop yield might be predicted from measurements. Characteristically, those who felt such models provided a useful insight into their problems reacted favourably, but others felt the degree of abstraction too far removed from current technology to be very useful.

Other topics covered included aspects of carbon metabolism and translocation, cell and apical growth, and rhythms and hormonal integration.

Although the papers varied considerably in the viewpoint which was taken this symposium provided something for everyone. The organisers are to be congratulated for providing an invaluable platform which was educational and extremely enjoyable for all concerned, and the published text is awaited eagerly. □

Miraculous peat?

from F. M. Slater

The 5th International Peat Congress was held in Poznan on September 21-25, 1976.

ONE interesting aspect of the Congress was the claim made for peat and peat preparations as a source of antiviral compounds, and of their use in the treatment of cancers, osteoarthritis and conditions arising from bronchial asthma. W. Adamek of Wroclaw, a medical practitioner, described 13 case histories of people with cancers of the skin, breast or alimentary canal who were treated using a peat extract developed by S. Tolpa (Academy of Agriculture, Wroclaw). Regression of the tumours was evident in ten of the patients when the preparation was administered orally (with intramuscular supplements in the case of breast cancer, rectal supplements for cancer of the lower bowel and poultices in the cases of skin cancer), and three reacted adversely to the treatments.

H. Wrobel and C. Jasiak (Janusz Korczak Hospital, Wroclaw) described how a peat preparation, named only as TK-, was beneficial in the treatment of hyperacidity which usually occurs in patients with bronchial asthma. Fifty patients were treated with the preparation which was administered orally before meals in doses of 30 g three times per day for 3 months. This treatment generally resulted in the complete disappearance or decrease of abdominal discomfort, disappearance

of distention, dull pains and unpleasant taste in mouth. Although the treatment obviously had a beneficial effect the authors admit that the mechanism of the therapeutic action remains unknown.

In much of Europe, unlike Britain, spas, peat baths and indeed the general study of balneology are still very much a feature of the medical scene, and K. Weber and G. Plötner of the German Democratic Republic presented the results of various peat bath therapies in the treatment of rheumatoid arthritis. They concluded that whatever peat consistency they used, all their patients benefited by a reduction in their erythrocyte sedimentation rate.

Although the successful treatment of foot-and mouth disease with peat litter containing humic acids has been demonstrated in the past, little recent work has been done on the antiviral properties of the humic acids of peat. R. Klöcking and M. Sprössig together with T. Klaus-Dieter (Medical Academy, Erfurt), however, presented a paper which demonstrated that ammonium humate possesses antiviral activity against herpes simplex virus types 1 and 2 when used in a concentration between 1 and $10 \mu\text{g ml}^{-1}$. The most humate-sensitive phase of virus multiplication seems to be the adsorption of viruses to the host cells. This report was supplemented by other work from the same institution by J. Witthauer, R. Klöcking, B. Helbig and P. Drabke who reported that the molecular weight of eluted humate was in the range 10^3 – 1.2×10^4 with a peak for ammonium humate at 3.3×10^3 . They also described a sensitive method for the detection of humic acids by means of polyacrylamide gel electrophoresis and staining with 0.2% alcian blue.

Although the congress went on to deal with peatland technology and conservation, it was the uses of peat for environmental protection which caught the imagination of delegates. Peat has been demonstrated to be an effective environmental filter for many substances. B. Illarionovich, G. Alexandrovna and C. Romanovna (Academy of Sciences, Minsk), described how peat's initial and natural affinity for elements such as Cu, Co, Zn, Ni, Ag and Ti can be increased 1.5 to 3-fold by previous oxidation of the peat with nitrogen tetroxide. Z. Rozmej and A. Kwiatkowski (Technical University, Gdansk) demonstrated the importance of brown coal in addition to peat as an ion exchange for metals including Al, Cr and perhaps most importantly U.

D. Asplund, E. Ekman and R. Thun (Technical Research Centre, Espoo) found peat to be effective in the puri-

fication of oily water. Untreated peat had an oil-binding capacity of 1–2 kg oil per kg peat, rising to 3.4 kg oil per kg peat when heat-treated peat was used.

In areas of high environmental value, such places as small camp sites and lay-bys may require a high purity of filtered wastewater such that very low nitrogen and phosphorous levels are present in the effluent. This demand combined with the usually low flow rates in such situations makes the wastewater filtration system described by J. L. Brown and R. S. Farnham (University of Minnesota, St Paul) the ideal answer. The basic design consists of 15 cm of coarse gravel covered by 50 cm of medium to fine sand topped by 30 cm of peat in which a crop cover is grown. The design can be modified by the addition of CaCO_3 to the peat to induce calcium phosphate precipitation or by an additional submerged peat layer to aid denitrification. A circular filter bed of this type 30 m in diameter would be adequate to treat the waste from 80 persons at the rate of 30 cm per week. Not only are effluent nitrogen and phosphorus very low but also, after three summers of operation, removal of total coliform bacteria remains nearly complete. $\square$

Itinerant-electron magnetism

from E. P. Wohlfarth

A conference on Itinerant-Electron Magnetism was held in Oxford on September 13–15, 1976. It was sponsored by the Magnetism Section of the European Physical Society and the Institute of Physics. The Proceedings will be published next Spring in the Europhysics Journal *Physica B*.

ALTHOUGH the birth of this subject took place some 40 years ago it was not until this September that a full scale conference was devoted to it. The meeting was concerned with the magnetic and related properties of metallic materials as influenced by their energy band structure, the interactions between the itinerant, that is, non-localised electrons, and the proper statistical mechanics determining thermal effects. This combination of influences leads inevitably to such a complex of physical problems that no progress is possible without the most judicious interplay between theory and experiment. Even 40 years turned out to be by no means too long a period to wait for an adequate summary of the status of this subject.

Several conflicting philosophies were

clearly revealed and their full resolution is just as clearly not yet at hand. One of these is concerned with the importance of spin fluctuation effects which T. Moriya (University of Tokyo) described. These effects give rise to serious modifications to the original Stoner model of itinerant magnetism which combines the molecular field approximation with Fermi statistics. D. M. Edwards (Imperial College, London) also discussed the importance of electron correlation effects in transition metals, and J. Hertz (University of Chicago) used an advanced many-body approach to discuss "sloppy" spin waves above the Curie temperature. Expressions of continuing support for the Stoner model in iron, nickel and cobalt were given by M. Shimizu (University of Nagoya). E. P. Wohlfarth (Imperial College, London) felt the case for nickel not proven but that another class of alloys, weak itinerant ferromagnets whose Curie temperature is low, continues to exhibit sufficient evidence for this model, especially where magnetoelastic effects are concerned. New measurements on this aspect were provided by G. Hilscher (Technical University of Vienna) and K. H. J. Buschow (Philips Research Laboratories, Eindhoven). On the other hand S. Ogawa (Electrotechnical Laboratory, Tokyo) and Y. Masuda (University of Nagoya) produced experimental evidence for the importance of spin fluctuations in describing dynamic properties of weak itinerant ferromagnets. M. Brodsky (Argonne National Laboratory) described evidence for related many-body effects in the specific heat of some actinide compounds.

A popular field of research in the subject concerns the spin polarisation of electrons emitted from ferromagnetic metals and alloys. R. Meservey (National Magnet Laboratory, MIT) described measurements showing that the polarisation of electrons tunnelling from alloys follows the static magnetisation closely. W. Eib (ETH, Zürich) showed new photoemission data for single crystal nickel specimens which showed an expected low energy sign reversal. A feeling thus arose, perhaps somewhat dangerously, that the ground state of nickel is fully understood using a simple band approach. M. Campagna (Bell Laboratories) also gave supporting evidence.

C. G. Windsor (Harwell) and Y. Ishikawa (Tohoku University, Sendai) described old and new data obtained by the most fruitful of all experimental techniques, involving neutron scattering. Among other results such measurements give valuable information on the properties of the elementary excitations in itinerant ferromagnets, the single particle and spin wave excitations.

Both of these were claimed by P. C. Riedi (University of St. Andrews) to have been observed by nuclear magnetic resonance measurements. A. J. Freeman (Northwestern University, Evanston) reported a wealth of calculations of neutron magnetic form factors and related quantities in metals.

Other magnetic materials whose properties may be discussed on the basis of an itinerant electron model include amorphous metals and alloys which were described by G. S. Cargill (IBM, Yorktown Heights) and M. J. Zuckermann (University of Paris, Orsay). R. D. Lowde (Harwell) and W. Young (Queen Mary College, London) gave a theoretical account of antiferromagnetic metals whose properties under stress were described by R. Griessen (Free University, Amsterdam) and E. Fawcett (University of Toronto), concentrating on chromium. More involved results on such materials, involving conductivity transitions, were described by J. M. D. Coey (CNRS, Grenoble).

Whereas the Hubbard Hamiltonian, discussed in its simpler aspects by M. Cyrot (CNRS, Grenoble) is a very use-

ful tool for describing the interactions between the itinerant electrons which are fundamental to the whole range of observed phenomena, an equally basic *sine qua non* are the energy bands themselves, both in pure metals and in alloys. New energy band calculations in nickel and iron were described by J. Callaway (Louisiana State University, Baton Rouge) who was able to estimate the parameter of the Hubbard Hamiltonian. Similar results were also obtained by O. K. Andersen (Technical University, Lyngby) and O. Gunnarsson (Institute of Theoretical Physics, Gothenburg). The approaches of these three talks present a new era of accuracy in band calculations as relevant to the magnetism of metals. J. Kanamori (University of Osaka), on the other hand, summarised the results of the coherent potential approximation in alloy calculations.

In the introduction to the Conference mention was made of the giants of the subject who were unable to be present. One of these, J. C. Slater, had only recently died and the Conference remembered his pioneering work with humility. □

Non-histone proteins

from Carol K. Klukas

THE complex composition of chromatin and the intricate interactions of DNA with histones and non-histone proteins (NHPs) which define its structure are of great interest to many biochemists today because these interactions are centrally involved in the system of control of eukaryotic genome expression. The innate complexity of chromatin structure has necessitated division of the general problem into smaller and more approachable segments. Isolated histones, for example, have been studied at great length including the sequencing of the individual proteins and more recently the analysis of the interactions of histones with each other and with DNA to form nucleosomes. Because the number of different histones is quite small and because their sequences have been highly conserved through evolution, the analysis of histones has been a relatively straightforward and manageable task. The non-histone proteins contained in chromatin, however, include many more individual species, which are each present in much lower quantities than the histones, and which, moreover, probably differ considerably with species and with tissue. From numerous *in vitro* transcription and reconstitution experiments it seems quite clear that the function of at least one or a few of the non-histone proteins is to direct exactly which of the genes are to be

transcribed from the DNA in a particular tissue at a specific time. The elucidation of how they interact with each other and with the histones and DNA to accomplish this task will be very difficult to be sure; however work in this direction has already begun as illustrated by a recent paper in *Biochemistry* (Gadski and Chae, *Biochemistry*, **15**, 3812; 1976).

In this paper Gadski and Chae present their studies of erythrocyte and reticulocyte chromatin dissociated in 2 M NaCl–5 M urea. The resultant reconstituted chromatin was analysed on acid-urea-polyacrylamide gels to determine which histones had bound to the DNA, as well as on SDS-polyacrylamide gels to determine which of the NHPs had bound. Using this assay system Gadski and Chae have found that at least one major high molecular weight NHP and two minor smaller molecular weight proteins remain associated with DNA in the 2 M NaCl–5 M urea dissociation conditions. Various other NHPs associate with DNA before, during and after the association of histones with DNA as the NaCl concentration is dropped. This indicates that the NHPs include protein species with a wide range of binding characteristics.

In similar experiments in which NHPs and DNA bound to cellulose were allowed to reassociate in the ab-

sence of histone, it was found that only 50% of reticulocyte NHPs bind to DNA in the absence of histone and NaCl, suggesting that some NHPs require histones for complete reassociation. While of the 50% of the NHPs which do bind in the absence of histone, the majority elute at 0.15 M NaCl, a significant fraction elutes only at lower NaCl concentrations at which the bulk of the histones do not bind to DNA. In general the NHPs which bind to free DNA have low molecular weights and do not show species specificity when compared to NHPs from Ehrlich ascites tumour chromatin.

Thus Gadski and Chae have begun to survey a range of characteristics of the individual proteins which comprise the NHPs of chromatin. Knowing the number of individual NHP species, their molecular weights, their relative concentrations and the conditions under which they will bind to DNA and/or to a DNA–histone complex will give clues as to the function of specific NHPs. Comparison of binding patterns such as presented here with those of the NHPs of other species might suggest which of the DNA-binding classes of NHPs are species specific and which thus might be likely candidates for key roles in the regulation of transcription. Obviously the type of experiment which Gadski and Chae present offers little information as to the specific



A hundred years ago

IN the very interesting Address delivered by Sir C. Wyville Thomson, at Glasgow, on the *Challenger* expedition, while referring to the "red clay" deposit so general over the deepest parts of the Atlantic and North Pacific, the remarkable fact is mentioned that the clay contains numerous nodules of peroxide of manganese, which in some places are found in great quantity. The Address goes on to say:—"This is a phenomenon which we are as yet unable to explain, and I do not know that there is any analogous instance in any of the older formations" (*NATURE*, vol. xiv, p. 494).

A GERMAN paper describes a dreadful fight between two Polar bears, male and female, in the Cologne Zoological Gardens. After a fierce struggle the female became exhausted, and was dragged by the male into the water basin in the den, and held down till life was quite extinct. He then pulled her out and dragged the body for a considerable time round the den. From *Nature*, **15**, November 16, 57, 70; 1876.

mechanism whereby the NHPs participate in control of genome expression, however, their results do certainly suggest some generalities about NHPs; such as that at least some of the NHPs must bind to the chromatin complex in cooperation with histones. Certainly many more studies must be performed using chromatin from different tissues and species before systematic comparisons among them all can be made. Such comparisons it is hoped, will begin to give clues as to which of the NHPs are responsible for control of the specificity of transcription and how each of them accomplishes this function. □

European geophysics

from a Correspondent

AFTER conferences in Zurich (1973) and Trieste (1974) the European Geophysical Society held its third meeting at the Free University of Amsterdam on September 7–10.

THE meeting attracted participants from both eastern and western Europe and the United States including a significant proportion of graduate students and 'younger' research workers.

In an effort to provide a forum for as many geophysical interests as possible, and to give the meeting a more open character, the scientific organisation was changed this year into a divided programme of eight convened symposia (containing some invited contributions) and seven more loosely structured subject sessions arranged on the basis of submitted abstracts.

In the symposium on Reliability of Palaeomagnetism: Criteria, Methods and Error Estimation several authors emphasised the importance of multi-component magnetisations, and there were few contributions which treated the problem from a purely rock magnetic standpoint. Among the most notable of these was that by H. Soffel (University of Munich) who showed the domain structure in pyrrhotite from a Devonian diabase. Using the Bitter technique and with the help of a Super-8 movie, Soffel demonstrated lamellae-shaped domains, the absence of closure domains, pseudo-single domain effects and the phenomena of domain wall pinning and magnetic viscosity. Soffel later used the same technique to justify the use of basalts with low Curie temperatures ($<70^{\circ}\text{C}$) in palaeomagnetic studies. Domain structure studies at different temperatures showed that the

largest titanomagnetite grains had the lowest Curie temperatures. Smaller grains and some grain margins had the highest Curie temperatures and it was these which carried the magnetically hard part of the natural remanent magnetisation.

The problem of multicomponent magnetisation in basaltic rocks was considered by R. Løvlie and K. M. Storetvedt (University of Bergen) with R. L. Wilson (University of Liverpool). Løvlie, in a paper read by Storetvedt, discussed Tertiary basalts from the Faeroes and questioned the general validity of the baked contact test for identifying primary magnetisations, suggesting that the Middle and Upper Lava Series could have been completely remagnetised in the Middle–Upper Tertiary times. In the Skye lavas Storetvedt showed that the large range of inclinations is due to a composite palaeomagnetic record and made the point that the original direction of magnetisation isolated in laboratory treatments is not always the most significant.

Many of the papers on sediments in the same symposium carried a similar theme. R. Thompson (University of Edinburgh) reviewed the criteria for assessing the reliability of palaeomagnetic data derived from unconsolidated sediments. He stressed the need for careful consideration of the physical and chemical factors which might affect the intrinsic magnetic character of unconsolidated sediments and emphasised the importance of chemical remanence. Thompson later went on to establish minimum criteria for establishing geomagnetic excursions by example from two cores from southern Sweden which spanned the time of the supposed Laschamp event (13,000–10,000 BP). Thompson showed that low inclinations and anomalous declinations were not repeatable between these cores and indicated that they were due to inwashed sands and older material.

Doubts were also cast on the validity of the intensity minima of the geomagnetic field during polarity transitions. Løvlie developed a model based on redeposition experiments of deep sea sediments which is compatible with a zone of gradual consolidation in which alignment and immobilisation depend on the grain size distribution of the magnetic particles. The implication is that the intensity minima associated with polarity transitions are due to some intrinsic character of the sediment rather than a weakening of the intensity of the geomagnetic field.

A symposium on The Sources of Marine Magnetic Anomalies: Nature and Location showed that interpretations regarding the source of marine magnetic anomalies are being re-

thought rapidly. The notion that the anomalies reside in the oceanic basalts which form oceanic layer 2A would seem to be an oversimplification and a much thicker source layer (perhaps the whole crust) seems likely. C. Harrison (University of Miami) and N. Watkins (University of Rhode Island) demonstrated that holes drilled into the Atlantic oceanic basement to a depth of a few tens of metres do not reveal consistent magnetisation. In particular there is much variation in inclination and the implication is that the source of the anomaly is distributed throughout the oceanic crust. W. Lowrie (University of Zurich) came to a similar conclusion from the Deep Sea Drilling Project. Results from 55 sites have inclinations which do not agree with expected values; also NRM intensity shows large between-site scatter and Lowrie concluded that layer 2A shows much lateral as well as vertical inhomogeneity.

Interesting results obtained by French research workers in Antarctica were described in the environmental geochemistry session. C. Boutron (University of Grenoble) presented data, collected at South Pole station, on heavy metal concentrations in snow samples which covered the period 1950–1974. He showed how concentrations of lead and copper had increased by factors of 2 and 3 respectively over this period (though no systematic trend was found for cadmium). While the results were in general agreement with aerosol data, the author stressed how dependent the enrichment determinations were on the reliability of the reference values adopted for the mean crustal composition. Caution was also expressed against attributing the high measured enrichments to global pollution alone; the influence of local sources requires full investigation also.

Some of the stabilising effects of partial melting of upwelling mantle material at ocean ridges were considered by E. R. Oxburgh and E. M. Parmentier (Oxford University) in a symposium on Kinematics and Dynamics of Plate Tectonics. By describing the sequence in which mantle material becomes depleted by removal of high density garnet, they were able to show that density differences between the starting material and the final residue were at least of the same order as density differences arising from the typical temperature variations which drive convective motions in the mantle. Although not proposing an alternative to the thermal convection mechanism for plate motion, Oxburgh pointed out that compositional density differences can be expected to have an important modifying role in determining the form of mantle circulation. □

review article

Observational constraints on the masses of neutron stars

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Direct information on the masses of five neutron stars is available at present. The mass estimates do not yet place any serious constraints on nuclear physics theory. However, if all five neutron stars have about the same mass, then the allowed mass range is quite narrow ($\sim 1.4\text{--}1.8M_{\odot}$) and is consistent with conventional pictures of neutron-star formation.

THE masses of neutron stars are of considerable theoretical interest, for two main reasons.

First, the demonstrated existence of a high mass neutron star would place constraints on nuclear physics, gravitation theory, or both. Nuclear physics in principle determines the equation of state of matter at extremely high densities. For a specific theory of gravity, the equation of state, in turn, governs the limiting mass of a stable neutron star. Following the epochal paper by Oppenheimer and Volkoff¹, much effort has been expended in estimating this limiting mass. When used to extrapolate the equation of state to densities well in excess of nuclear-matter densities ($\sim 2 \times 10^{14} \text{ g cm}^{-3}$), conventional theories of many-body nuclear physics, in conjunction with the theory of general relativity, predict a limiting mass in the range $1.7\text{--}2.7M_{\odot}$ (refs 2–4). However, a limiting mass as large as $\sim 3\text{--}5M_{\odot}$ is still consistent with all direct experimental nuclear and high energy physics data^{2,5,6}. It has been shown^{7,8} that differential rotation cannot increase the limiting mass by more than $\sim 30\%$. However, if one modifies the theory of gravity, a stable neutron-star model of much larger mass can be constructed^{8,9}.

Second, in the most widely accepted picture of stellar evolution^{10,11}, a highly evolved massive star develops a degenerate core that grows by accretion of mass from the surrounding stellar envelope. As the mass of the core approaches the Chandrasekhar limiting mass ($\sim 1.4M_{\odot}$) appropriate to its composition, a thermonuclear runaway and/or dynamical collapse ensues, presumably leading to a supernova event and, in at least some cases, the production of a neutron-star remnant. Although there are many remaining theoretical uncertainties (see, for example, refs. 12 and 13), a neutron star that is formed in this manner may well have a mass comparable to that of the core of the progenitor star (that is, $\sim 1.4M_{\odot}$). If a large fraction of observed neutron stars are actually found to have masses near this value, then substantial support for conventional pictures of advanced stellar evolution and neutron-star formation would have been obtained.

In the past few years, significant empirical information on the masses of several neutron stars has become available for the first time. These neutron stars are all in binary systems, and the mass information is derived from the observed interactions between the neutron stars and their companions. In this paper, we describe the present state of empirical knowledge of neutron star masses and discuss the theoretical implications of this information.

Binary systems containing neutron stars

At present, there are four known binary systems that contain X-ray pulsars and one system that contains a radio pulsar. (Six other X-ray pulsars have been discovered^{14–17} and these objects are also likely to be in binary systems, but neither conclusive evidence of binary membership nor useful orbital information is yet available.) For three of the binary pulsars, the pulse period (P) is well under 2 s, which is too short to be a plausible surface rotation period of a white dwarf (see for example, ref. 18) or the pulse period of a white dwarf that is vibrating in its fundamental mode¹⁹. Moreover, the existence of periodic pulsations rules out the possibility that any of these objects is a black hole. Thus, all three of these pulsars are almost certainly neutron stars. The remaining two objects (the X-ray pulsars 3U0900–40 (ref. 20) and Cen X-3 (refs 21, 22)) are also almost certain to be neutron stars, but the reasons for this conclusion are somewhat more complex and will be explained below.

Of course, it is also very likely that the remaining radio pulsars are all neutron stars. However, the neutron stars in binary systems are unique in that it is possible to place significant constraints on their masses that are independent of any theoretical assumptions concerning the equation of state of matter at high densities.

The existence of an object that emits periodic pulsations in a binary system enables one to measure the period, P_{orb} , and projected semimajor axis, a_p , of the orbit of the pulsar, from which one can infer the mass function:

$$f(M) = \frac{4\pi^2 (a_p \sin i)^3}{GP_{\text{orb}}^2} = \frac{M_c^3 \sin^3 i}{(M_p + M_c)^2}$$

where i is the inclination of the orbital plane with respect to the plane of the sky, M_p is the pulsar (neutron star) mass, and M_c is the mass of the companion star. To derive useful constraints on M_p , it is necessary to obtain independent information concerning the values of M_c , i , or both. The nature of this information is different for each of the five binary systems of interest.

In the following paragraphs, we describe the relevant observational properties of each of the five binary systems, the type of information used to constrain the value of M_p , and the indicated range of allowable values of M_p . Quoted error bars and confidence limits are taken from the original observational work, and wherever possible we state the level of significance. In all cases, the final limits on M_p are not formal confidence limits, but are rather the minimum and maximum values that are consistent with all of the available observational information.

3U0900–40 is an eclipsing binary system, as are all four

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X-ray pulsar binaries, with $P_{\text{orb}} \simeq 8.966$ d (ref. 23). The binary is composed of an X-ray star and an optical companion, HD77581 (ref. 24), that has been classified as B0.51b (ref. 25). It has been discovered²⁰ that the X-ray star is a long period pulsar, with $P \simeq 283$ s, $a_p \sin i = 111.4 \pm 3.3$ (1 σ) light s, and $f(M) = 18.46 \pm 0.79$ (1 σ) $M_\odot$ (ref. 26). The orbit was also found to be slightly eccentric, with eccentricity $e \simeq 0.13$ (ref. 26).

The apparent brightness of HD77581 ($V \simeq 6.9$; ref. 27) makes it relatively easy to measure the optical radial velocity variations due to orbital motion. Hence, this system can, in effect, be treated as a double-line 'spectroscopic' binary. The optical radial velocities can be used to derive the projected semimajor axis ($a_c \sin i$) of the companion star, and one thereby obtains a second relation for the stellar masses in terms of measured quantities

$$M_p(a_p \sin i) = M_c(a_c \sin i)$$

Unfortunately, there are large non-statistical fluctuations in the optical radial velocities, probably caused by variations in the strong stellar wind from HD77581 (ref. 23) or other mass flow within the binary system. The smallest value of $a_c \sin i$ that has appeared in the literature is based on an analysis²⁶ of the most recent optical data obtained by van Paradijs *et al.*²⁸: $a_c \sin i = 7.79 \pm 0.74$ (1 σ) light s. On the other hand, the orbital fit to optical data that gives the largest value of $a_c \sin i$ and is still consistent with the known orbital eccentricity (which was recently determined accurately from X-ray data)²⁶ is solution 1a by Wallerstein²⁹: $a_c \sin i \simeq 15.1$ light s.

The final constraints that one uses are the limits on $\sin i$ that can be derived from analysis of the ellipsoidal light variations of the companion star (which are caused by its rotation and its tidal distortion by the X-ray star) and the X-ray eclipse duration (which is also affected by the rotational and tidal distortion). The observed value of the eclipse duration is 1.90 ± 0.05 d (ref. 30). The most recent analyses^{31,32} indicate that $70^\circ \lesssim i \lesssim 90^\circ$ if $a_c \sin i \simeq 7.8$ light s, and $80^\circ \lesssim i \lesssim 90^\circ$ if $a_c \sin i \simeq 15.1$ light s. We note that one of these analyses³¹ correctly gave the range of acceptable values of $a_p \sin i$ before the measurement of this parameter from X-ray data, which lends credence to the standard theoretical picture of X-ray binary systems that is used in this method of analysis and to the results of similar analyses of other X-ray binaries described below.

Small values of $a_c \sin i$ and large values of i correspond to small values of M_p , and vice versa. Combining the measured mass function with the probable lower and upper limits on i and $a_c \sin i$, we obtain for the range of allowable values of M_p

$$1.4M_\odot \lesssim M_p \lesssim 3.5M_\odot$$

M_p is probably greater than the maximum stable mass for a white dwarf in the absence of differential rotation (internal rotation periods $\lesssim 10$ s; surface rotation period $\simeq 283$ s), and such strong differential rotation would probably induce a dynamical instability (compare refs 18, 33). For these reasons it has been concluded^{20,26} that this slow pulsar, as well as the fast pulsars discussed below, is almost certainly a neutron star.

The X-ray source Cen X-3, with $P \simeq 4.842$ s, was the first X-ray pulsar to be discovered^{21,22}. The highly circular orbit ($e \lesssim 0.003$ (ref. 34)) of the X-ray star has $P_{\text{orb}} \simeq 2.087$ d (ref. 22), $a_p \sin i = 39.73 \pm 0.03$ light s (ref. 22), $f(M) = 15.46 \pm 0.04 M_\odot$ (ref. 22), and an X-ray eclipse duration of 0.45 ± 0.02 d (refs 35, 36). A B0II star was established by Krzeminski³⁷ as the optical companion. The pulse period is not quite short enough to rule out a white dwarf model for this X-ray star, but its very high X-ray luminosity ($\sim 3 \times 10^{37}$ erg s⁻¹ (ref. 38)) strongly rules against white dwarf models³⁹. Hence, this object is again almost certain to be a neutron star.

When the measured ellipsoidal light variations^{37,40} of Krzeminski's star and the X-ray eclipse duration are incorporated into theoretical models for the tidal and rotational distortion of the optical star^{36,41,42}, the mass ratio ($q = M_p/M_c$) and i can be estimated: $0.04 \lesssim q \lesssim 0.09$ and $74^\circ \lesssim i \lesssim 90^\circ$. The acceptable values of q and i are strongly correlated. When these

constraints are combined with the measured mass function, limits on M_p are obtained^{36,41,42}

$$0.6M_\odot \lesssim M_p \lesssim 1.8M_\odot$$

Situated in the Small Magellanic Cloud, SMC X-1 is the only extragalactic X-ray source known to be in a binary system⁴³. The optical companion, Sk160 (refs 44, 45), is of spectral type BOI (ref. 44). Recently, pulsations from the X-ray star with $P \simeq 0.715$ s were discovered⁴⁶, followed shortly thereafter by a measurement of the orbital Doppler curve of the pulsar⁴⁷. The nearly circular orbit ($e \lesssim 0.04$ (ref. 47)) has $P_{\text{orb}} \simeq 3.892$ d (ref. 48), $a_p \sin i = 53.83 \pm 0.35$ (1 σ) light s (ref. 47), $f(M) = 11.05 \pm 0.22$ (1 σ) $M_\odot$ (ref. 47), and an X-ray eclipse duration of 0.60 ± 0.03 d (refs 43, 47, 48).

The projected orbital velocity amplitude ($v_c \sin i$) of Sk160 has not been well determined, but the available measurements indicate that $v_c \sin i \sim 40$ km s⁻¹ (ref. 49), corresponding to $a_c \sin i = (P_{\text{orb}}/2\pi) v_c \sin i \sim 7$ light s. Taking 20 and 60 km s⁻¹ as probable lower and upper limits on $v_c \sin i$, and using the observed X-ray eclipse duration in conjunction with theoretical models of the tidal and rotational distortion of the optical star to place constraints on the permissible values of i at each value of q , one obtains⁴⁷

$$1.1M_\odot \lesssim M_p \lesssim 4.0M_\odot$$

The ellipsoidal light variations of Sk160 have been measured⁵⁰⁻⁵⁴ but their analysis is complicated by the effect of X-ray heating of the optical star, which is appreciable in this system. Estimates of q and i based on such analyses^{55,56} are consistent with those obtained from the considerations described in the preceding paragraph.

Hercules X-1 is unique among all X-ray binaries in that the companion star, HZ Herculis⁵⁷⁻⁶⁰, is known to be of relatively late spectral type. When the X-ray star is in eclipse, the spectral type is late A or early F; but when the X-ray star is visible, the optical luminosity increases by a factor of ~ 4 and the spectral type changes to B (refs 58, 61-65). These optical variations are apparently caused by the strong heating of one face of the optical companion by the X-ray star. Unfortunately, the uneven surface brightness greatly complicates the extraction of mass information from the measured optical radial velocities, since these velocities are consequently affected strongly by the stellar rotation as well as the orbital motion^{62,66}.

The strong X-ray heating also precludes observation of the ellipsoidal light variations at the present time. However, from photographic plates dating back to 1890, it has been found⁶⁷ that the X-ray star apparently ceases its emission for extended periods lasting months to years, during which HZ Her displays ellipsoidal variations. The number and quality of the old photographic plates are insufficient to place useful constraints on q and i (refs 42, 66), but observations of future inactive-state ellipsoidal variations should be very powerful in this regard.

The binary system has $P_{\text{orb}} \simeq 1.700$ d (refs 68, 69), a nearly circular orbit ($e \lesssim 0.002$; ref 34) and an X-ray eclipse duration of 0.24 ± 0.01 d (ref. 69), and the X-ray pulsar has $P \simeq 1.238$ s (ref. 68, 69), $a_p \sin i = 13.18 \pm 0.03$ light s (ref. 68), and $f(M) = 0.85 \pm 0.01 M_\odot$ (ref. 88). Estimates of M_p have been made⁶⁶ using this eclipse duration and mass function together with theoretical models of the tidal and rotational distortion of HZ Her. One obtains $q \lesssim 0.7$, no meaningful constraint on i , and

$$M_p \lesssim 1.8M_\odot$$

Other methods of estimation of M_p give values that are consistent with this range but are unable, at present, to constrain more strictly the range of allowable values^{42,66}.

Recently, another independent estimate of M_p has been carried out⁷⁰, based on an analysis of extensive observations of 1.24-s optical pulsations that are occasionally seen from the system and apparently result from the reprocessing of pulsed X rays near the surface of HZ Her. In the context of the

assumed model, it was found that HZ Her must fill its critical lobe and a value of $M_p = 1.30 \pm 0.14 M_\odot$ was thereby obtained. However, it is uncertain at present how sensitive this result is to some of the assumptions of the model, including the assumption that HZ Her is in synchronous rotation and the implicit assumption that there is no strong orbital-phase-dependent obscuration of the optical pulsations by other matter within the binary system. At least one further analysis of the optical pulsations is being carried out at present (T. Chester, personal communication).

PSR1913+16 is the only radio pulsar (of ~ 150) that is known to be in a binary system. The pulsar, which has $P \simeq 0.0590$ s, was found to be in an eccentric binary orbit with $e \simeq 0.62$, $P_{\text{orb}} \simeq 0.3230$ d, $a_p \sin i \simeq 2.336$ light s, and $f(M) \simeq 0.1313 M_\odot$ (refs 71, 72). No eclipses are observed in this system⁷¹.

During subsequent observations over the course of about a year, it was discovered that the longitude of periastron (related to the angle between the view direction from the earth to the star and the line of closest approach from the star to its companion) was changing at a rate of $\dot{\omega} = 4.22 \pm 0.04^\circ \text{ yr}^{-1}$ (ref. 72). This effect, known as apsidal motion, can be completely accounted for by general relativity. However, it is possible, depending on the radius and other properties of the unseen companion, that some or all of the apsidal motion could be caused by a tidally or rotationally induced quadrupole moment of the companion^{73,74}. Under the assumption that the observed apsidal motion is entirely a general relativistic effect, the measured mass function can be combined with the general relativistic expression for this effect to obtain a relation for the sum of the two stellar masses^{72,75}

$$M_p + M_c = 2.83 M_\odot$$

If some of the apsidal motion is due to a tidally induced quadrupole moment, or to a rotationally induced quadrupole moment with the axis of rotation at least $\sim 35^\circ$ out of the orbital plane⁷⁶, then $M_p + M_c$ must be less than this value.

A normal main-sequence star would induce a value of $\dot{\omega}$ much larger than that observed^{73,76,77}. The companion could be a white dwarf, neutron star, or black hole, or it might be a peculiar star that is nondegenerate but nonetheless has an anomalously small radius ($\lesssim 0.1 R_\odot$). Many theoretical studies of such stars have appeared in the literature (see, for example, refs 78–81).

Combining the sum of the masses with the measured mass function, one obtains constraints on the allowable values of M_p (ref. 75). If the companion is a white dwarf, then its mass must be less than $\sim 1.4 M_\odot$ (unless it is undergoing rapid rotation) and this yields $1.4 M_\odot \lesssim M_p \lesssim 1.9 M_\odot$. In the case where the unseen companion is also a neutron star, then $M_p \lesssim 1.9 M_\odot$ and the mass of the companion neutron star is also constrained: $1.0 M_\odot \lesssim M_c \lesssim 2.9 M_\odot$. Moreover, at least one of the two neutron stars must have a mass $\gtrsim 1.4 M_\odot$. For other possible types of companion stars, all that can be determined is that $M_p \lesssim 1.9 M_\odot$.

In summary, as long as the theory of general relativity is correct, one obtains:

$1.4 M_\odot \lesssim M_p \lesssim 1.9 M_\odot$ for a normal white-dwarf companion;
 $M_p \lesssim 1.9 M_\odot$ for other types of companion.

Summary and conclusions

At present, there are five X-ray and radio pulsars that are very probably neutron stars and that are contained within binary systems. The periodic pulsations from these pulsars and their interactions with their respective companion stars enable constraints to be placed on the neutron-star masses. The available constraints are summarised in Fig. 1.

It is apparent that these mass estimates are all still entirely consistent with the predictions of nuclear physics. None of these neutron stars need have a mass greater than $\sim 1.4 M_\odot$, while a minimum mass of $\sim 1.8 M_\odot$ would be needed to exclude at least

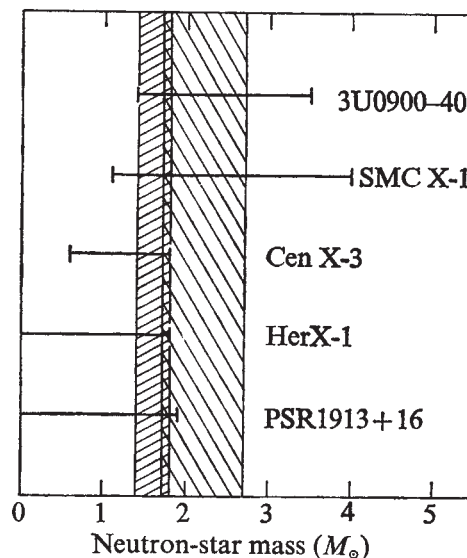


Fig. 1 Empirical constraints on the masses of neutron stars. For each of the five binary systems containing neutron stars, the bar denotes the range of masses that is consistent with all of the available observational information. The close-hatched region ($1.4\text{--}1.8 M_\odot$) represents the range of masses consistent with all five neutron stars. The open-hatched region ($1.7\text{--}2.7 M_\odot$) indicates the range of limiting neutron-star masses predicted by present conventional theories of many-body nuclear physics (see text).

some of the equations of state that have been derived²⁻⁴ from present conventional theories of many-body nuclear physics.

On the other hand, if one hypothesises that most or all neutron stars have about the same mass, then the range of masses that are allowable for all five cases is quite narrow ($\sim 1.4\text{--}1.8 M_\odot$; Fig. 1). It is noteworthy that this is consistent with the range of masses that one might expect if neutron stars are formed in supernova events resulting from the collapse of the degenerate cores of highly evolved stars.

For 3U0900–40, Cen X-3 and SMC X-1, significant refinement of the neutron-star mass estimates must probably await extensive new observations of the optical radial velocities and an improved theoretical understanding of systematic fluctuations in the radial velocities and other measured properties of the companion stars. In the case of Her X-1, further analysis of the optical pulsations is promising as an improved method of mass estimation. In addition, when Her X-1 enters another extended X-ray inactive state, measurements of the radial velocities and ellipsoidal light variations of the optical companion should greatly refine the mass estimate of the neutron star. Further refinements of the neutron-star mass estimate of PSR1913+16 will be possible when additional mass-dependent relativistic effects (gravitational redshift and second-order Doppler shift) can be separated from the first-order orbital Doppler shift; this will occur when the longitude of periastron advances through an appreciable angle from its present value, which will require $\gtrsim 5$ yr (ref. 82).

Several other X-ray pulsars have recently been discovered and it seems likely that most or all of these objects are in binary systems. For at least two of these objects, 3U0352+30 and A0535+26, there is already some direct observational evidence of binary membership^{83–85}. As additional pulsars are discovered and shown to be in binary systems, and as constraints on their masses are developed, the improved observational statistics will constitute a further refinement in empirical information on neutron-star masses.

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articles

Polarisation detection at radio wavelengths in three spiral galaxies

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Polarisation at radio wavelengths has been measured in the disk radiation of M51, M81 and M31 using the Westerbork Synthesis Radio Telescope. The magnetic field of M51, as implied by the polarisation vector map, is oriented preferentially in the tangential direction. The intrinsic polarisation of the disk radiation of M51 is estimated to be, on average, 12%, corresponding to an anisotropy (or degree of uniformity) in the magnetic field of 14% with scale length ≤ 3 kpc. A 7% lower limit is set on the disk polarised radiation of M81, but on some parts of the disk of M31 it reaches 20%.

WE have constructed a model which is especially suitable for the interpretation of radio polarisation measurements on the disks of spiral galaxies—polarisation is evidence for anisotropy in the magnetic field. Models which relate, under various conditions of field topology and object geometry, the observed linear polarisation and a measure of the anisotropy of the field have been constructed by Korchakov and Syrovatskii¹ and by Burn², but neither of these models is quite applicable to the case we discuss, although, for the conditions of interest, the results do not seem to be very model dependent.

We shall consider a region in a disk galaxy defined, of necessity, by the angular resolution of the observations (see Table 1). This region is thought of as divided into volume elements of equal size, so small that the magnetic field within

each element is essentially homogeneous and unidirectional. We now represent the distribution of magnetic fields by supposing that a fraction f of the volume elements contains a field with a single uniform spatial orientation ($\mathbf{u}$), while the orientation of the field in the remaining elements is randomly distributed ($\mathbf{r}$). We further assume that the distributions of relativistic electron densities and magnetic field strengths are the same in both components. If this is not the case, then the contributions to the observed intensity from the two components must be weighted according to their mean volume emissivities, $\langle n_{\text{rel}} H^{(\gamma+1)/2} \rangle$, where γ is the electron energy spectral index. The fraction f is a measure of the anisotropy of the magnetic field within the resolution element. Using standard expressions³, it is easily shown that under the above conditions the degree of intrinsic polarisation is

$$\rho = \frac{3\gamma+3}{3\gamma+7} \left[1 + \frac{(1-f)\pi^{1/2}\Gamma[(\gamma+5)/4]}{2f(\sin \theta)^{(\gamma+1)/2}\Gamma[(\gamma+7)/4]} \right]^{-1} \quad (1)$$

where θ is the angle between the line of sight and $\hat{\mathbf{u}}$.

The observations described in this paper indicate that the orientation of $\mathbf{u}$, in M51 at any rate, is largely tangential in the galactic plane. We shall make only the more general assumption that $\mathbf{u}$ is parallel to the disk and, taken over the galaxy as a whole, has no further preferential orientation with respect to

and i is the angle between the plane of the sky and the galactic plane. In Fig. 1, $\langle \rho \rangle$ is shown as a function of f for $\gamma = 2.5$ and for two values of i .

Two points should be stressed: first, the degree of anisotropy, f , refers to the angular resolution element. We expect that it will increase with decreasing beam size; second, no account has been taken of depolarisation from either differential Faraday rotation along the line of sight or from the changes in rotation measure with position within a resolution element. This will often be of importance, particularly in regions of high density, and thus the value of the anisotropy derived by this method will be a lower limit. At $\lambda = 6$ cm, however (see below), we do not expect Faraday depolarisation to be very effective.

Observations and results

The WSRT has been used to observe M51, M81 and M31. The telescope and reduction procedures are described elsewhere⁴⁻⁸. The observational parameters for each of the maps presented hereafter are given in Table 1. The system noise at 1,415 MHz has been reduced by a factor of ~ 2.5 since earlier observations of the same objects with the WSRT.

M51

M51 has been observed with the WSRT at wavelengths of 6, 21 and 49 cm. No polarisation was detected at 49 cm implying

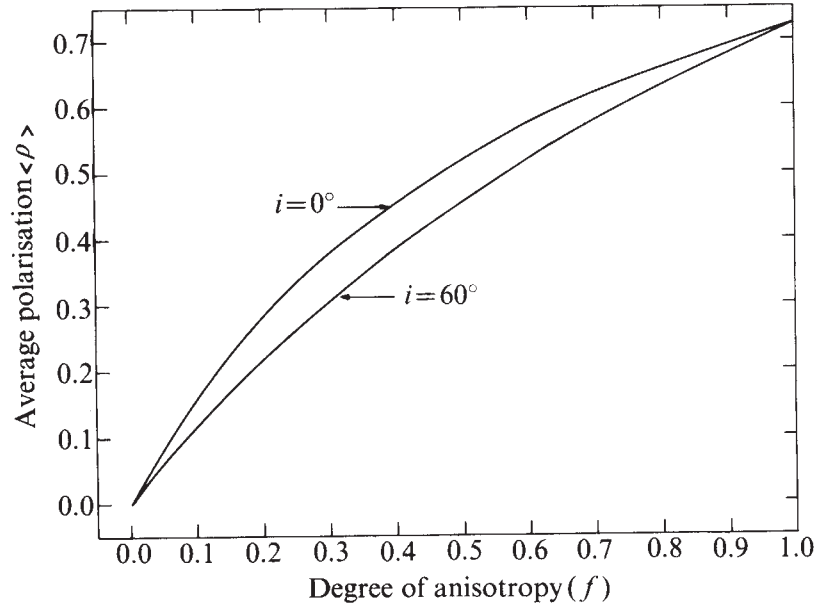


Fig. 1 The average intrinsic degree of polarisation, $\langle \rho \rangle$, plotted as a function of the degree of anisotropy of the magnetic field, f , for electron energy spectral index $\gamma = 2.5$ and two values of the galactic inclination, i .

any fixed direction in space. Clearly a tangential field belongs to this class. In this case, the average degree of polarisation (the scalar sum of the polarised flux from all resolution elements divided by the total flux) is given in equation 1 with $(\sin \theta)^{(\gamma+1)/2}$ replaced by its average value

$$\langle \rho \rangle = \frac{3\gamma+3}{3\gamma+7} \left[1 + \frac{(1-f)\pi^{1/2}\Gamma[(\gamma+5)/4]}{2f\Gamma[(\gamma+7)/4]F(i)} \right]^{-1} \quad (2)$$

where

$$\begin{aligned} F(i) &= \frac{1}{2\pi} \int_0^{2\pi} (1 - \sin^2 i \sin^2 \phi)^{(\gamma+1)/4} d\phi \\ &= 1 + \sum_{n=1}^{\infty} \frac{(-1)^n}{n! 4^n} \sin^{2n} i \prod_{j=1}^n \frac{(\gamma+5-4j)(2j-1)}{2j} \end{aligned} \quad (3)$$

a 3σ upper limit on the polarised brightness temperature of 1.5 K. The beam HPBW of the synthesised 49-cm beam is $56'' \times 76''$, or 2.6×3.6 kpc at an assumed distance of 9.7 Mpc (ref. 9).

Polarisation at 6 cm

The 6-cm observations of M51 have been described elsewhere¹⁰. To increase the signal to noise ratio, the observations were convolved to a $56'' \times 76''$ beam and two maps were produced: an intensity map and a polarisation map (Fig. 2). No appreciable Faraday rotation is expected at such a short wavelength in view of the residual polarisation which is observed at 21 cm (see below). We find it significant, therefore, that at a level $> 2.5\sigma$ ($\sigma = 0.009$ K = 0.9 mJy/beam) the polarisation vectors are generally perpendicular to the spiral arms. This suggests an approximately tangential magnetic field structure. For the Galaxy, such a field configuration is referred to as 'longitudinal' (as opposed to helical). In a recent paper,

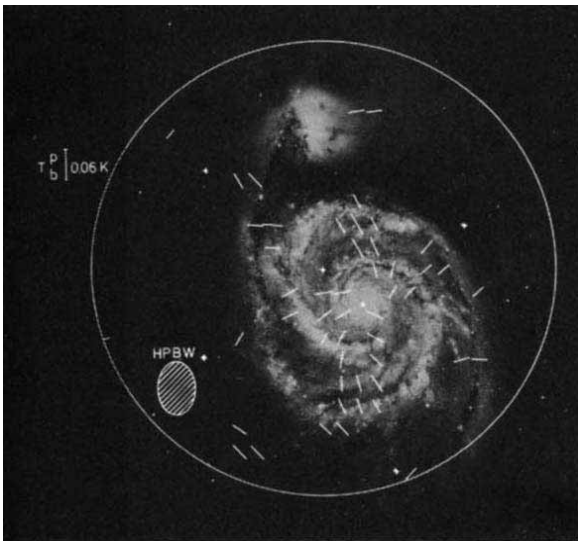


Fig. 2 The polarisation vectors of M51 at 6 cm. Vectors whose amplitude is < 2.5 mJy per beam ($\sim 2.5\sigma$) are not drawn. The polarised flux is not corrected for the primary beam attenuation. The primary beam half power circle is drawn as a continuous line.

Spoelstra and Brouw (in preparation) argue that a longitudinal field also fits the radio polarization observations of the Galaxy better than a helical field. A tight-helix field configuration would result in a net polarisation along the spiral arm. Such a configuration is ruled out by our results. Our observations do not exclude, though, a very-open-helix field configuration.

The location of the polarised brightness peaks, which range up to ~ 0.05 K, cannot be precisely determined because of the large beam and the weak signal. The percentage polarisation at a few points was computed by dividing the polarised flux by the intensity deduced from the preliminary MPIfR 100-m dish observations of M51 at 4.8 GHz (Wielebinski, private communication). Peaks as high as 20% were found. To determine the average polarisation, we have analysed the distribution of the observed polarised signal to derive the polarised flux (the scalar sum over all resolution elements) corrected statistically for noise. A similar correction has been

applied to all polarisation data. The corrected polarised flux was divided by the total flux within the same region after subtracting a point source of 15 mJy at the nucleus. We find an average polarisation of 12% for radii < 9 kpc (limited by the off-centre instrumental polarisation). Because of the short wavelength, this is probably characteristic of the intrinsic polarisation. This implies an anisotropy of the magnetic field, f , of 14% on the scale of the resolution element (see Fig. 1; the inclination of M51 is taken to be 35°).

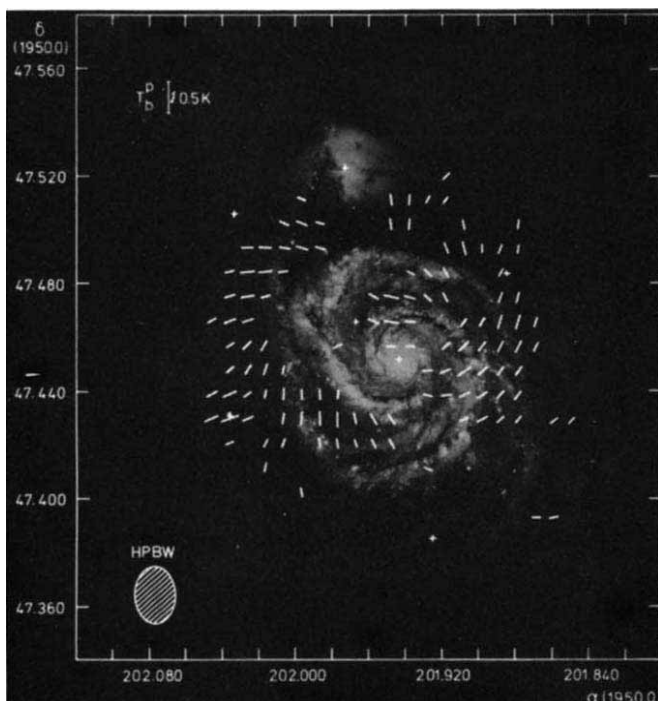


Fig. 3 The polarisation vectors of M51 at 21 cm. Vectors whose amplitudes are < 1 mJy per beam (0.12 K $\approx 2.5\sigma$) are not drawn.

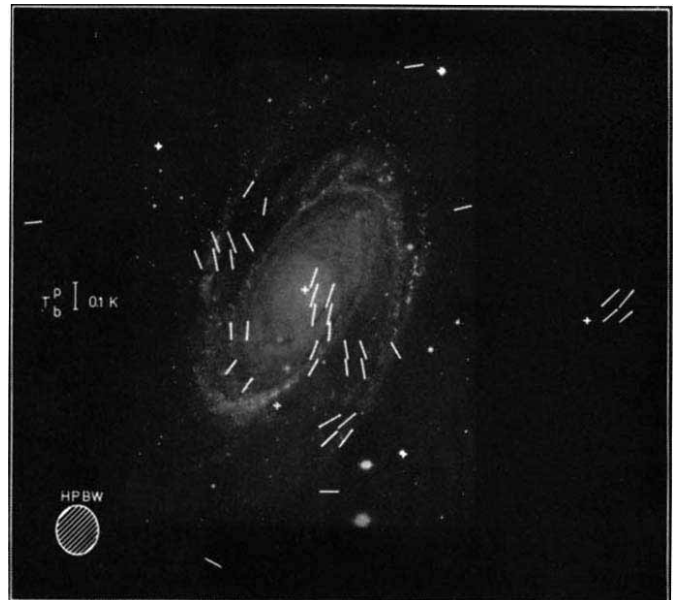


Fig. 4 The polarisation vectors of M81 at 21 cm. Vectors whose amplitudes are < 1.3 mJy per beam (≈ 0.05 K $\approx 3\sigma$) are not drawn. The polarised flux is not corrected for the primary beam attenuation. The polarisation vectors at the western edge of the photograph are related to an external source and may partly arise from instrumental polarisation.

Polarisation at 21 cm

New 21-cm observations of M51 have recently been carried out with the WSRT and will be described elsewhere (Segalovitz, in preparation). Polarisation at the 3σ level ($\sigma = 0.25$ mJy/beam $= 0.17$ K, determined empirically) was detected at a few scattered locations in the full resolution map ($\text{HPBW}_\alpha \times \text{HPBW}_\delta = 24'' \times 32'' = 1.1 \times 1.5$ kpc). These peaks did not in general coincide with regions of maximum intensity. They are several times weaker than the polarisation peaks reported previously¹¹.

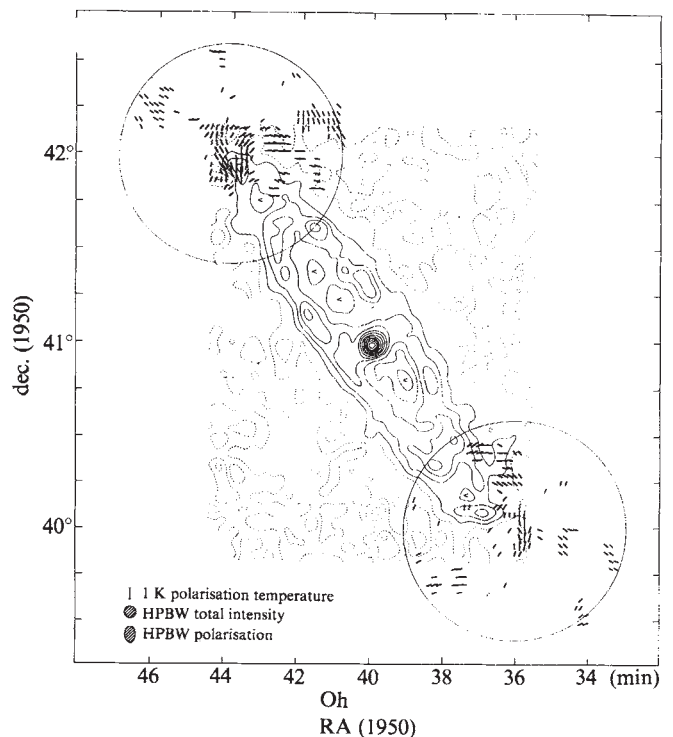


Fig. 5 Polarisation vectors at 49 cm in two fields in M31. Only amplitudes > 0.34 K ($\approx 5\sigma$) have been plotted. The two circles correspond to a primary beam attenuation of 0.67. With few exceptions, only points lying inside these circles have been plotted. The amplitudes have not been corrected for primary beam attenuation. The polarisation vectors are shown superposed on the 408 MHz total intensity map of Pooley¹⁵. The contour interval is 3 K, corresponding to ~ 1 K at 610 MHz, and the outermost solid curve is the first contour above the nominal zero level. Because of the missing short spacings, the true zero level is uncertain.

Convolving to a $56'' \times 76''$ beam (and thus increasing the signal-to-noise ratio) revealed an extensive distribution of polarised radiation (Fig. 3). The polarised flux over a large part of the Galaxy is at a general level of 1.25 mJy/beam ($= 0.15$ K $\approx 3\sigma$) with peaks at the 0.21-K level. The strongest peak (0.27 K) is associated with an external source E of M51. About half of the area from which the polarised radiation comes is not located at intensity peaks, and for this the resolution is not good enough to make a definite statement. The tendency of the polarisation peaks to be found away from the intensity peaks may result from a less ordered magnetic field or stronger Faraday depolarisation in regions of higher density. The same tendency is found in strong extragalactic radio sources^{12,13}.

In most regions the polarisation vectors are systematically rotated by $\sim 45^\circ$ counterclockwise from the radial direction. Vallée and Kronberg¹⁴ estimate a galactic Faraday rotation in the direction of M51 of -10 ± 15 rad m^{-2} , corresponding to a clockwise rotation of $25 \pm 37^\circ$ at 21 cm. This estimate is not consistent with our data (from comparison of the directions of the 6-cm and 21-cm polarisation vectors). Small-angle deviations of this magnitude from the values predicted from their model are not, however, unexpected. We conclude, from the uniformity of the polarisation vector rotation, that the

systematic Faraday rotation over the resolution element in M51 is at most of comparable magnitude to that estimated in the Galaxy ($\lesssim 20$ rad m^{-2}).

A polarisation map was made by dividing the polarised flux by the total intensity at points where the polarised flux was above the 3σ level. Polarisation peaks reach values of 20%. The average polarisation percentage was derived by dividing the total polarised flux by the total intensity, and was found to be 2.5% for radii < 12 kpc. In this calculation, the flux of the nucleus of M51 (35 mJy) was first subtracted from the total flux. Comparing this value with the apparently intrinsic 12% polarisation, we get a rough estimate of 50 rad m^{-2} for the r.m.s. variations in rotation measure on angular scales much smaller than the resolution element of $56'' \times 76''$ (using the model of Burn²) as compared with the upper limit of 20 rad m^{-2} for large scale fluctuations.

M81 at $\lambda = 21$ cm

The disk of M81 has been observed with the WSRT at 21 cm and at 49 cm. A detailed account of the observations will be given elsewhere (Segalovitz, in preparation). The 49-cm observations show no polarisation > 2.5 K (3σ). The 21-cm

Table 1 Instrumental and observational parameters

Galaxy	M51	M51	M81	M31
Wavelength (cm)	6.0	21.2	21.2	49.2
1 K is equivalent to. . . (mJy per beam)	103	8.24	27.9	19
R.M.S. noise* (mJy per beam; K)	0.9; 0.0088	0.4; 0.48	0.44; 0.016	1.2; 0.06
Observing dates (day/month/year)	25-27/12/74	7,21/6/75	10,19/6/75	15,20,25,26/11/75
	1-6/1/75	5/7/75	30/7/75	3,5,9,10/12/75
	8,17/1/75	7/8/75	3,19/8/75	
Observing time (h)	12 $\times$ 12	4 $\times$ 12	4 $\times$ 12	2 $\times$ 4 $\times$ 12
Baseline coverage: from, to, by (m)	36,180,18	36,612,18	36,306,18	36,378,18
Primary beam, HPBW†	11	36	36	83
f.w.h.m. of synthesised and restoring beam (RA'' $\times$ dec'')	56 $\times$ 76	56 $\times$ 76	114 $\times$ 126	214 $\times$ 326
HPBW of synthesised and restoring beam (linear size in the galactic plane, kpc)	3.2 $\times$ 3.6	3.2 $\times$ 3.6	3.6 $\times$ 2.0	2.4 $\times$ 1.0
Scale corresponding to shortest baseline†	5.7	20.2	20.2	47
Position of field centre (RA(°); dec(°))	201.9666; 47.4666	201.9666; 47.4666	147.92; 69.22	9.0; 40.0 and 11.0; 42.0†
No. of figures in this paper	2	3	4	5

*The values refer to the Q, U and I maps.

†Centres of two fields (see text).

observations convolved to a $1.9' \times 2.1'$ beam (1.8×2 kpc at a distance of 3.2 Mpc) reveal polarisation at a level of 0.05 K to 0.07 K ($\sigma = 0.016$ K = 0.44 mJy per beam) as shown in Fig. 4. The polarisation direction shows no preferential orientation with respect to the azimuthal direction at the few points where it can be determined. Polarisation percentages were derived by dividing the polarised flux by the intensity at some points where a comparatively high polarised flux was detected. The total flux densities were taken from the corresponding intensity map after adding a constant 0.035 K to correct approximately for flux in large scale features which is not detected by the Westerbork telescope. Peaks at the 20% level were found. The average polarisation, derived by dividing the total polarised flux by the total flux after subtracting the nucleus, was 7%. This is considerably higher than for M51. The difference may arise from a smaller Faraday depolarisation in M81 or to a more ordered magnetic field. Taken at face value, the average polarisation of 7% implies that 10% of the magnetic field is ordered (see above and Fig. 1; the inclination of M81 is taken to be 59°). Since Faraday depolarisation inside the Galaxy may be appreciable at 21 cm, the above value should be regarded as a lower limit.

M31 at $\lambda = 49$ cm

A survey of M31 at $\lambda = 49$ cm (see Table 1) has recently been completed. The results will be published in full elsewhere. Examination of the first reductions shows widespread linear polarisation, and a sample of these preliminary results, derived from two fields centred on the major axis and $80'$ on either side of the nucleus, is illustrated in Fig. 5. (Observations of the central region have not yet been fully reduced.) The polarisation vectors have been derived from maps convolved to a $3.6' \times 5.4'$ beam. Only vectors whose amplitudes are $\geq 5\sigma$ (see first note in Table 1) have been plotted. Since the maps of total intensity, whose preparation is much more elaborate on account of the multitude of background sources and the missing short spacings, are not yet available, the polarisation vectors have been superimposed on Pooley's 408-MHz ($\lambda = 73$ cm) map of M31 (ref. 15), which corresponds to approximately the same angular resolution ($4' \times 4'$). Assuming a spectral index of $\alpha = 0.8$ ($S \propto \nu^{-\alpha}$), the contour interval of 3 K on the 408-MHz map would become ~ 1 K on a corresponding map at 610 MHz. Pooley's observations were carried out with the E-vector

of the feed in position angle 90° and, depending on the polarisation, may not be representative of the unpolarised intensities at specific points. Moreover, because of the missing short intervals, the nominal zero may correspond to a true brightness temperature of as much as one contour interval above zero. Three other radio continuum surveys¹⁶⁻¹⁸, at various frequencies and resolutions, all indicate typical brightness temperatures at 49 cm of 1–2 K in these regions.

As in the case of M51, the regions of maximum polarised brightness temperature do not generally coincide with peaks of intensity. While the maximum degree of polarisation is as yet difficult to estimate because of the uncertainty in the zero level, it must amount to several tens of per cent in some regions. Even where the total intensity is relatively high, and correspondingly less uncertain, degrees of polarisation $> 20\%$ are found. A quantitative discussion of these results must, however, await complete reduction of the WSRT observations. Although we might expect quite large Faraday rotation at this frequency, the polarisation vectors show considerable consistency in orientation locally and tend to maintain a constant orientation with respect to the spiral pattern. This suggests that for the regions of M31 where polarisation is detected, the internal Faraday rotation is small compared to M51 and M81.

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Cytoskeletal control of surface membrane mobility

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Cytochalasin B induced redistribution of actin microfilaments into a bulge of the cytoplasm, bringing cell-surface elements to the corresponding surface area. This process was reversible, inhibited by colchicine and enhanced by antibodies to cell-surface antigens.

THE mechanisms responsible for the organisation of the surface membrane¹ and for the mobility of molecules within it are not understood. Long- but not short-range movements of surface antigens²⁻⁸ are dependent on cell metabolism^{2,9,10}, and inhibited by cytochalasin B (CB)^{7,11}, but not by colchicine¹¹. CB is thought to disrupt a micro-

filament system, containing actomyosin, located below the surface membrane¹². It has therefore been proposed that redistribution of cell surface molecules depends on microfilament activity². In most studies, however, CB has affected membrane mobility only partially^{2,7,10,11} and since the compound has many other effects it may influence membrane mobility in several ways not necessarily involving microfilaments. In addition, CB induces morphological effects on the surface membrane, suggesting that this is one site of action of the compound¹¹.

Microtubular activity has been linked with ligand-induced redistribution (capping) of membrane components in lymphocytes. Capping of immunoglobulin (Ig) on mouse lymphocytes is inhibited by concanavalin A (con A) in

large doses. This inhibition is abolished by colchicine¹⁵⁻¹⁷, which disrupts microtubules. Colchicine also augments the inhibitory effect of CB on capping¹¹. Con A-capping in rabbit ovarian granulosa cells redistributes cytoplasmic microtubules¹⁸.

Here we present evidence for an interaction of cell-surface molecules with the submembranous microfilament system of actomyosin and with the microtubules. In three test systems CB induced a simultaneous reversible redistribution of cell-surface molecules and submembranous actin microfilaments which was counteracted by colchicine.

The cells studied were uninfected, and measles virus (Edmonston strain)-infected heteroploid human lung fibroblasts of the strain Lu 106, mouse L-cells, mouse

lymphocytes, normal human lymphocytes and cells of two human lymphoblastoid cell lines of B cell origin. The antigenic specificities examined included measles virus haemagglutinin (HA), H-2, β_2 -microglobulin (β_2 -m), species antigens (rabbit anti-Lu 106), and Ig on the appropriate cell types.

CB-induced redistribution

Cells were first incubated for 60 min in spinner culture in Eagle's spinner medium supplemented with 5% foetal calf serum (FCS), and then for a further 60 min in the presence of CB ($10 \mu\text{g ml}^{-1}$) unless otherwise stated. Thereafter, to

Fig. 1 *a*, Untreated Lu 106 cells. Indirect IF-staining detecting β_2 -m. *b*, Lu 106 cells treated with CB. Polarisation of blebs. Indirect IF-staining detecting β_2 -m. *c*, Lu 106 cells treated with CB plus colchicine. Blebs distributed all around the cell surface. Indirect IF-staining detecting β_2 -m. *d*, Lu 106 cells treated with CB. Discontinuous IF-staining detecting β_2 -m. Indirect technique. *e*, Chronically infected Lu 106 cells treated with CB. Caps. Indirect IF-technique detecting measles virus haemagglutinin. *f*, Chronically infected Lu 106 cells kept in 0.1% DMSO. Virtually continuous IF-staining. Indirect IF-technique detecting measles virus haemagglutinin.

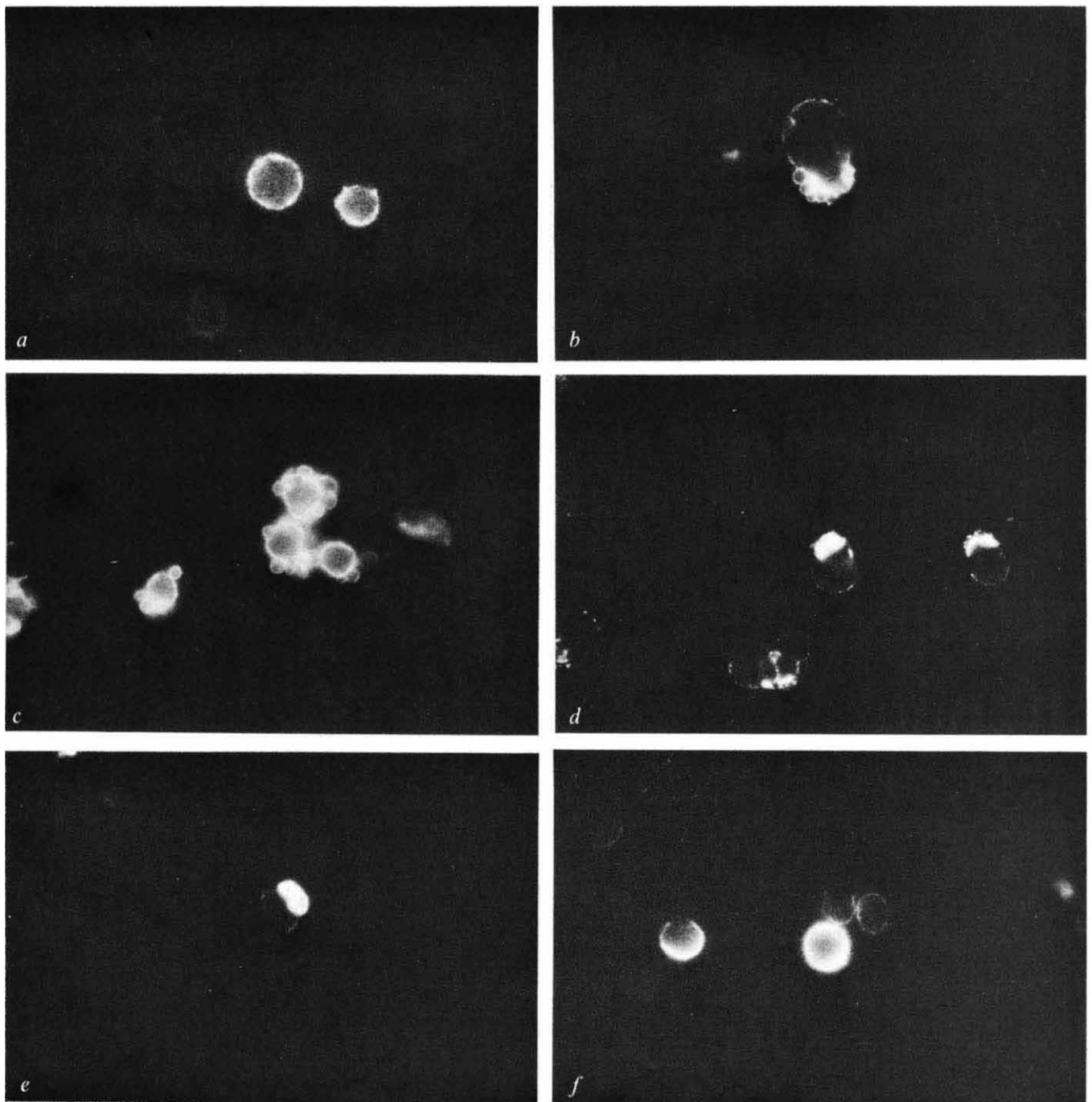


Table 1 *a* Effects of cytochalasin B on cell morphology

Cells	Antiserum against	Treatment					
		None	CB	CB + Colchicine	Colchicine	Antibodies	CB + Antibodies
Carrier	Ha	Round cells	IF-neg blebs at uropods	IF-neg blebs around the cell	Round cells	Round cells	IF-neg blebs at uropods
	β_2 -m	Round cells	IF-pos blebs at uropods	IF-pos blebs around the cells	No blebs	No blebs	IF-pos blebs at uropods
	Species antigens	Round cells	IF-pos blebs at uropods	IF-pos blebs around the cells	No blebs	No blebs	IF-pos blebs at uropods
Lu 106	β_2 -m	Round cells	IF-pos blebs at uropods	IF-pos blebs around the cells	Round cells	Round cells	IF-pos blebs at uropods
	Species antigens	Round cells	IF-pos blebs at uropods	IF-pos blebs around the cells	No blebs	No blebs	IF-pos blebs at uropods
L-cells	H-2	Round cells	IF-pos blebs at uropods in 70% of cells	Uropods in 30% of cells (partial inhibition)	Round cells	Round cells	No blebs

b Effects of cytochalasin B on antigen distribution at the cell surface

Cells	Antigen	Treatment					
		None	CB	CB + Colchicine	Colchicine	Antibodies	CB + Antibodies
Carrier	HA	Confluent	Caps	Confluent	Confluent	Discontinuous, caps	Pronounced capping
	β_2 m	Confluent	Discontinuous, caps	Confluent	Confluent	Discontinuous, few caps	Pronounced capping
	Species antigens	Confluent	Discontinuous	Confluent	Confluent		
Lu 106	β_2 m	Confluent	Discontinuous, caps	Confluent	Confluent	Discontinuous	Pronounced capping
	Species antigens	Confluent	Discontinuous	Confluent	Confluent		
L-Cells	H-2	Confluent	Discontinuous, polarised to the uropod	Confluent	Confluent		

The cells were incubated for 60 min at 37 °C in Eagle's spinner medium supplemented with 5% FCS before further treatment. The cells, 2×10^6 cells in 1 ml per tube, were then incubated further for 60 min *a*, with no treatment; *b*, in the presence of $10 \mu\text{g ml}^{-1}$ of CB; *c*, in the simultaneous presence of $10 \mu\text{g ml}^{-1}$ of CB and 10^{-2} M of colchicine (colchicine was added 30 min before CB); *d*, in the presence of 10^{-2} M colchicine, and subsequently PFA-fixed before IF-staining. When antibodies were present during the experiment, the cells were reacted with antiserum and conjugate, respectively, for 30 min + 30 min before CB—incubation in 1 ml at 37 °C for 60 min, followed by PFA-fixation. Carrier cells and Lu 106 cells were stained with a rabbit anti-HA serum, a rabbit anti- β_2 -microglobulin serum, a rabbit anti-HeLa serum, a monkey anti HeLa serum, respectively. The L-cells were stained with mouse ACA anti-A/SN and C57BL anti-C3H, respectively.

prevent further redistribution, cells were fixed in 4% paraformaldehyde for 10 min at 4 °C. Finally, antigens were detected on the surface membrane by direct or indirect immunofluorescence staining for 30 min or 30+30 min respectively, at 0–4 °C.

The morphology of Lu 106 and L-cells was affected by CB (Fig. 1, Table 1*a*). Cells exposed to CB often formed a bulge which looked like a uropod¹⁸ (Fig. 1). These were most frequent on L-cells, occurring in 80% of cases. In addition, cells treated with CB had 5–15 blebs on the part of the cell carrying the "uropod". β_2 -m and species-specific antisera reacted strongly with the blebs whereas the HA-specific antiserum did not react.

The distribution of antigens at the cell surface was also affected by CB (Fig. 1, Table 1*b*). After incubation in the presence of CB the measles virus HA was present as "caps" on the "uropods" of 40% of infected cells. Control cells incubated without CB, or in the presence of 0.1% DMSO, had a ring-type distribution of HA at the cell surface (Fig. 1). β_2 -m and species antigens of both infected and uninfected Lu 106 cells were also redistributed, less, however, than the HA, and some of these antigens were unaffected.

In CB-treated L-cells, reacted with anti-H-2 sera (ACA anti-A and C57BL anti-C3H/K), cell surface antigens were redistributed in the direction of the "uropod". The extent of redistribution was similar to that of β_2 -m and species antigens on Lu 106 cells. Attempts to induce redistribution of surface antigens by CB in normal lymphocytes of mouse and human origin and in human lymphoblastoid cell lines failed.

Concentrations of CB between 1 and $5 \mu\text{g ml}^{-1}$ were necessary to achieve movement of HA on measles-infected cells, and above $10 \mu\text{g ml}^{-1}$ redistribution reached a plateau (Table 2). While $5 \mu\text{g ml}^{-1}$ was enough for some cap formation of HA, redistribution of β_2 -m required $10 \mu\text{g ml}^{-1}$.

To see whether the induced redistribution of antigens was reversible, CB-treated cells showing redistribution were kept at 37 °C in the absence of CB for various times, and then fixed and reacted with antisera against HA and β_2 -m. After 10 min the number of "uropod"-carrying cells with redistributed surface antigens had decreased from 40 to about 10%. After a further 50 min antigens were again homogeneously distributed.

Tables 1*b* and 3 show the influence of antibody plus colchicine on CB-induced redistribution of cell surface antigens. Measles-virus infected and uninfected Lu 106 cells incubated in the presence of 0.5×10^{-2} M colchicine and CB at $10 \mu\text{g ml}^{-1}$ showed "capping" of β_2 -m in 3% of all cells whereas 15% exposed to CB alone showed "capping". The number of cells exhibiting CB-induced polar distribution of measles virus HA was decreased in the presence of 10^{-2} – 10^{-3} M colchicine (Table 3). Colchicine, however, did not inhibit the formation of blebs. In its presence they were distributed all over the surface membrane (Fig. 1, Table 1*a*). So colchicine seemed to interfere with the movement of blebs to the "uropod".

Simultaneous exposure of infected and uninfected Lu 106 cells to antibody and CB, respectively, increased the frequency, rate and degree of redistribution of surface antigens significantly compared with exposure to each alone

Table 2 Kinetics of redistribution of haemagglutinin (HA)

A		B		C			
Treatment with cytochalasin B for 60 min ($\mu\text{g ml}^{-1}$)	Cells with capped HA (%)	Treatment with cytochalasin B at 10 $\mu\text{g ml}^{-1}$ Time (min)	Cells with capped HA (%)	Cells staining with trypan blue after treatment with DMSO 0.1 %	No treatment	Cytochalasin B 10 $\mu\text{g ml}^{-1}$	
0	0	0	4 3	0	4.7 5.6		
1	0	10	13 15				
5	13	30	28 25	60	3.0 4.7	5.3 5.7	3.0 4.0
10	41	60	44 31				
				120	3.0 5.7	6.0 7.3	3.0 5.0
100	41	120	41 43				

At least 200 cells were counted when percentage of cells was calculated. In *B*, two individuals calculated one set of values separately (A. E. upper and K.-G. S. lower value). In *C*, duplicate tubes were set up and the counting performed by one person. The experiments were performed with carrier cells, trypsinised and resuspended in Eagle's spinner medium supplemented with 5% foetal calf serum and kept in 37 °C for 60 min before the beginning of the experiments. Cytochalasin B was dissolved in DMSO and 10 $\mu\text{g ml}^{-1}$ of CB corresponds to 0.1 % DMSO. The cells in *A* and *B* were fixed with 4% PFA before IF staining with anti-HA serum and examination.

(Table 1b). Thus, at a time when antibody-induced redistribution alone had not yet occurred, in the presence of both CB and antibodies, all antigens studied exhibited pronounced redistribution. This synergistic effect seemed to be independent of which of the reagents, the antibody or CB, were added to the cells first.

Cocapping of surface antigens and microfilaments

The behaviour of submembranous microfilaments of actin in relationship to the redistribution of surface components was examined in uninfected Lu 106 cells. Cells were suspended in 0.034 M sodium citrate, smeared on glass, fixed in dry acetone at -20 °C for 15 min and stained with a human serum containing antibodies to actin.

Before CB treatment, or when treated with DMSO used to dissolve CB, the cells showed virtually continuous IF-staining of their margin and a diffuse staining of the cytoplasm when reacted with anti-actin serum (Fig. 2). In contrast, CB-treated cells exhibited a redistribution of actin to the uropod which was completely reversible within 30 min (Fig. 2). This indicated that the redistribution of actin, and of cell surface antigens described above, were related to each other. To study this further double IF-staining with separate fluorochromes for actin and cell surface antigens was performed. The microfilament system was detected with the human actin antiserum²⁰ and a rhodamine (TRITC)-conjugated sheep anti-human IgG, absorbed with rabbit serum. Cell-surface antigens (β_2 -microglobulin, species-specific antigens) on Lu 106 cells were detected using the appropriate rabbit antisera and a fluorescein (FITC)-conjugated sheep anti-rabbit IgG, absorbed with normal human serum. Before CB treatment, the cells showed continuous IF-staining of their margins with both anti-actin serum (rhodamine) and antisera to surface antigens (fluorescein). On CB-treated cells the rhodamine staining was localised in the cytoplasm corresponding to the "uropod" but in many cells the redistribution was incomplete. To document the results further we took advantage of the synergistic influence of CB and antibody on the redistribution of cell surface molecules. This demonstrated cocapping of surface antigens and actin to the "uropod" (Fig. 3).

The specificity of the anti-actin serum was checked by absorption with purified actin²⁰. This clearly decreased the number of cells that stained with rhodamine as well as the

IF-intensity of the cytoplasm. Anti-actin serum did not stain viable Lu 106 cells.

Mechanisms of cytoskeletal control

Our principal observations were the coincident reversible CB-induced redistribution of microfilaments and cell-surface elements into a bulge of the cytoplasm and the antagonistic and synergistic influence of colchicine and antibodies, respectively, on this process. These results strongly support: (1) the existence of a connection, direct or indirect, between cell surface elements, microfilaments and microtubules; (2) the concept that cytoskeletal structures influence the behaviour of molecules in the plane of the membrane^{15-17,21}; and (3) the concept that microfilaments and microtubules are responsible for the cellular control of membrane mobility and integrity.

It is uncertain, however, whether microfilaments and microtubules antagonise or synergise in provoking and/or preventing membrane mobility. Cytochalasins have been suggested to affect the microfilament system by either disruption¹² or induction of contraction²². Therefore, interpretation of the results in terms of the function of microfilaments and microtubules depends on which of these two effects is favoured. It seems unlikely that the observed effects of CB on the cell surface¹⁴ are responsible for the redistribution of cytoplasmic microfilaments, unless these were exposed on the cell surface. Also it seems unlikely that the redistribution of cell-surface elements, so well correlated to the redistribution of microfilaments, should be due solely to a surface effect of CB. It should be re-

Table 3 Effect of colchicine on CB-induced redistribution of measles virus haemagglutinin

Experiment*	Treatment	Capped cells (%)
1	CB 10 $\mu\text{g ml}^{-1}$	39
	CB 10 $\mu\text{g ml}^{-1}$ + colchicine 10 ⁻² M	2
	CB 10 $\mu\text{g ml}^{-1}$ + colchicine 10 ⁻³ M	5
	CB 10 $\mu\text{g ml}^{-1}$ + colchicine 10 ⁻⁴ M	9
2	CB 10 $\mu\text{g ml}^{-1}$	30
	CB 10 $\mu\text{g ml}^{-1}$ + colchicine 10 ⁻² M	4
	CB 10 $\mu\text{g ml}^{-1}$ + colchicine 10 ⁻³ M	10
	CB 10 $\mu\text{g ml}^{-1}$ + colchicine 10 ⁻⁴ M	7
3	CB 10 $\mu\text{g ml}^{-1}$ + colchicine 10 ⁻² M	0
	CB 10 $\mu\text{g ml}^{-1}$ + colchicine 10 ⁻³ M	6
	CB 10 $\mu\text{g ml}^{-1}$ + colchicine 10 ⁻⁴ M	9
	CB 10 $\mu\text{g ml}^{-1}$ + colchicine 10 ⁻⁵ M	14

*Experiment 2 was performed in the presence and experiments 1 and 3 in the absence of FCS.

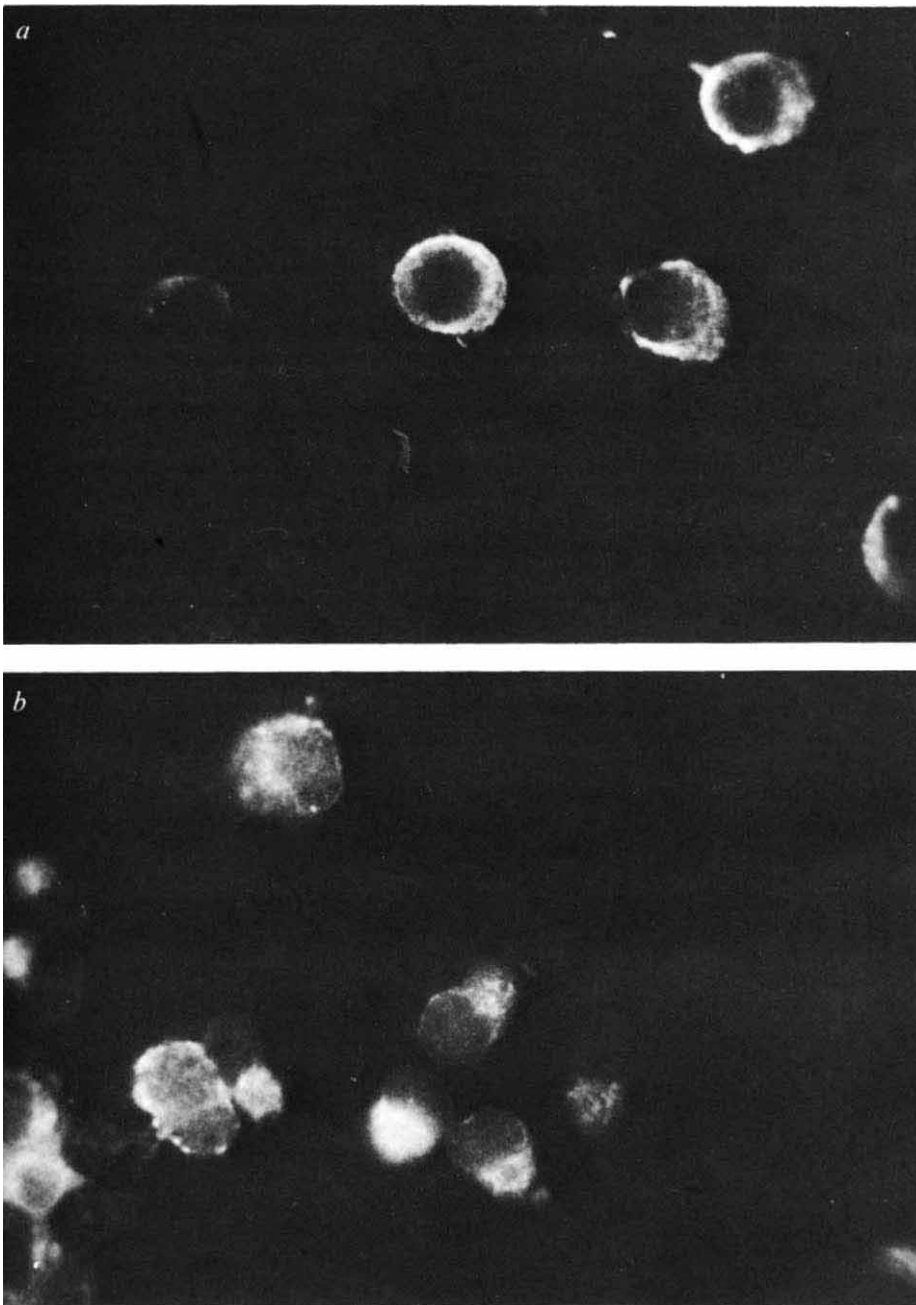


Fig. 2 Distribution of actin in uninfected Lu 106 cells *a*, before and *b*, after CB-treatment. Notice the redistribution of IF-staining to uropods.

membered that antibody-induced redistribution of cell surface antigens did not redistribute microfilaments in the absence of CB. Attempts to redistribute microfilaments by capping cell surface antigens in various test systems have failed (K.-G.S., unpublished observations). The reason for this may be that cross linking of cell surface antigens by antibody involves only microfilaments corresponding to the antigens studied, whereas CB redistributes more of the microfilaments, as the present data suggest.

It is interesting in the light of our result that microfilaments in some cell types contract after exposure to CB (ref. 22). Furthermore, cytochalasins induce the formation of knobby protuberances and the migration of these at the surface membrane^{23,24}. The protuberances resemble the blebs we obtained after treatment with CB.

The degree of redistribution varied for different antigenic specificities and cells as reported before with antibody-induced redistribution²⁵⁻²⁷. Measles virus HA exhibited the

greatest redistribution. It was present only on the capped area of the cell surface. HA was absent from the blebs whereas the other specificities were found along the margins of these protrusions. Thus, there may be a correlation between exclusion from blebs and a pronounced tendency to redistribution. This, together with the earlier findings^{7,25-27}, suggests that certain structures in the cell surface membrane, such as HA, are more dependent on the microfilament system than other membrane components.

Our results may seem difficult to reconcile with the observations that CB alone or with colchicine inhibits capping of lymphocytes¹¹ and that colchicine enhances capping in lymphocytes treated with con A (ref. 15). These discrepancies may be related to relative variations in the localisation, structure, quantity or association to membrane elements of microfilaments and microtubules in different cell types. This is supported by the absence of CB-induced redistribution in certain cell types in the study reported

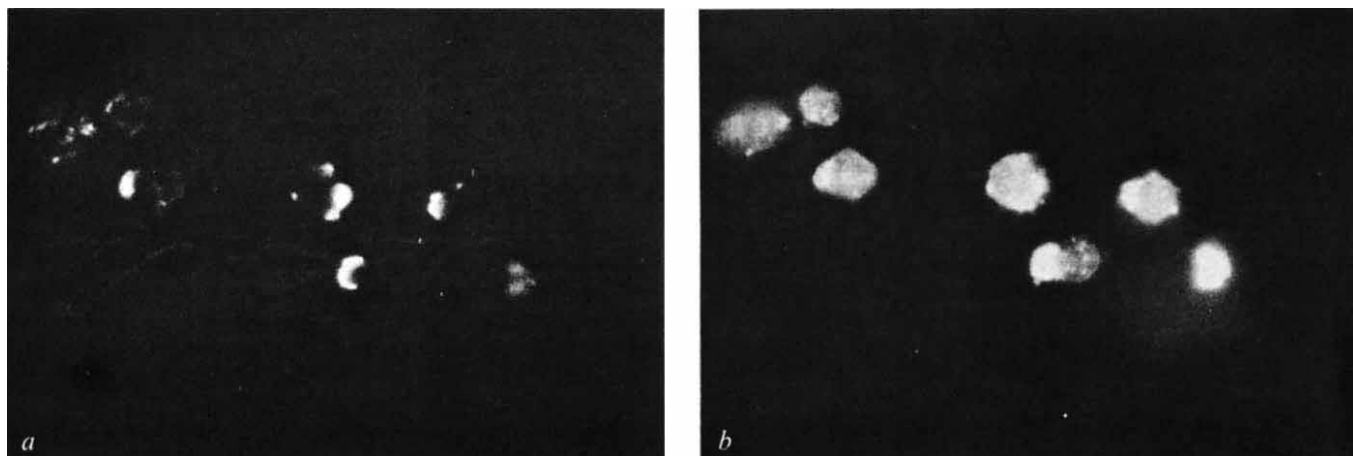


Fig. 3 Cocapping of β_2 -m and actin in CB-treated Lu 106 cells. *a*, Distribution of β_2 -m detected by indirect FITC-staining; *b*, Distribution of actin detected by indirect TRITC-staining. The live cells were first stained by rabbit anti- β_2 -m, diluted 1:20 and an absorbed sheep anti-rabbit IgG, diluted 1:5, 30 min, 0 °C respectively. Subsequently, CB 10 μ g in 1 ml was added and the cells incubated 60 min and then acetone-fixed. Actin was demonstrated by staining with a human anti-actin serum diluted 1:20 and an absorbed sheep anti-human conjugate diluted 1:2.5.

here. Such differences could conceivably influence both the nature and the magnitude of the cellular response to the two compounds. Thus, it is important to determine when CB and colchicine, respectively, induce and antagonise membrane mobility and to characterise the cellular basis for this difference between different cell types.

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X-ray diffraction studies of circular superhelical DNA at 300–10,000-Å resolution

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X-ray diffraction studies on circular superhelical DNA from bacteriophage PM2 at a very low scattering angle show that it is possible to measure the superhelical scattering function of the molecule. The results suggest that in addition to the primary supercoil, a higher order of supercoiling of the DNA is present, an effect which can be interpreted with a simple analogue.

THE supercoiling of circular double helical DNA, first demonstrated by Vinograd, has been studied by various physical and

chemical techniques¹. If the superhelix turns are fairly uniformly distributed, the periodicity should give rise to maxima in the X-ray diffraction pattern, the position of which depends on the precise geometry of the superhelix. Depending on the correct value of the superhelix density (which has been in dispute for some time) and on the addition of unwinding agents such as intercalating molecules (for example, ethidium bromide), the diffraction maxima are expected in the range from several thousand Angstroms down. Accordingly, we have explored the low angle diffraction from dilute solutions of circular superhelical DNA from PM2 bacteriophage² from about 10,000 Å down to about 300 Å. Two significant maxima are observed. The first, due to the "native" superhelix, appears at angles corresponding to a Bragg spacing of 337 Å. The second is observed at 2,100 Å, increasing with the addition of ethidium bromide (EB), in a manner to be expected if the latter unwinds

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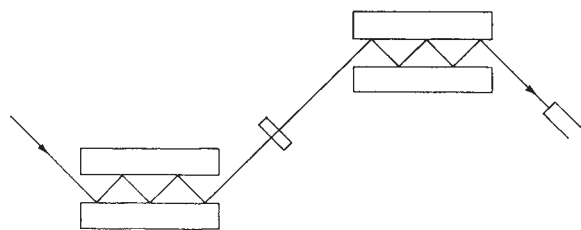


Fig. 1 Schematic drawing of the Bonse-Hart design for low angle X-ray scattering.

the helix. These results suggest that in addition to the primary supercoil, a higher order of supercoiling of the DNA is also present, an effect which can be interpreted with a simple analogue. A more general conclusion is that with the methods described here it is possible to observe the helix in an environment approaching that of *in vivo* conditions.

The DNA used in these studies was prepared according to the methods of Espejo *et al.*² from the bacteriophage PM2, which was grown and purified using modifications of previously published methods³⁻⁵. We verified that the intact DNA was in the closed circular supercoiled conformation by sedimentation velocity studies in alkaline and neutral CsCl and by Agarose gel electrophoresis. Nicked samples were prepared as required with pancreatic DNase.

The DNA usually had an absorbance ratio at 260–280 nm of 1.95–2.0. When examined in alkaline and neutral CsCl in the Beckman Model E ultracentrifuge using a Vinograd band-forming cell, only one band was visible. The DNA was further concentrated, when required, by vacuum evaporation. By this method, the solution could be reduced to about 1/100 of the original volume. After concentration, it was again dialysed against buffer (0.1 M NaCl, 0.2 M Tris (pH 7.5), 1 mM disodium EDTA), frozen quickly in a dry ice-acetone mix, and stored at -20°C . The DNA concentration used in the experiments was 2 mg ml^{-1} , at 0.1 M salt concentration.

Two sets of X-ray measurements were made. Those for scattering angles greater than $300\text{ s } 2\theta$ were taken with a Kratky camera using $\text{CuK}\alpha$ radiation; those in the scattering range

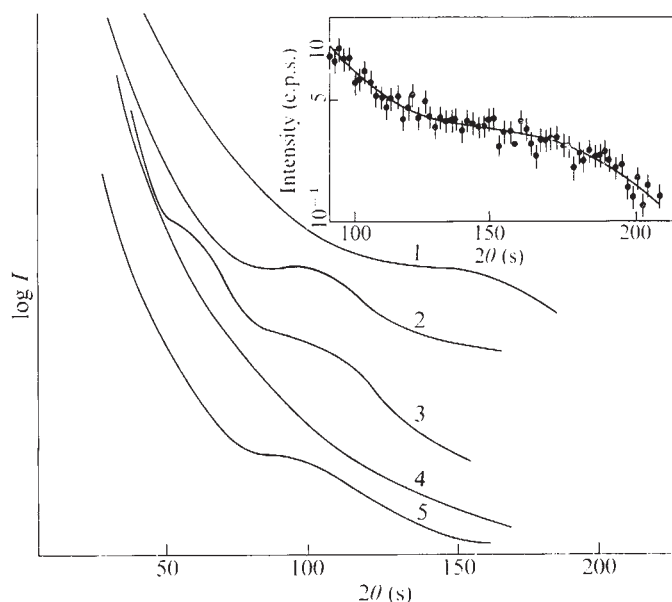
from 12–300 s were made with a small-angle apparatus constructed after the principle first demonstrated by Bonse and Hart⁶. The Kratky camera and its operation are well known⁴ and we need not discuss further the technique of that set of measurements. The Bonse-Hart apparatus is more unusual. In this arrangement two grooved germanium single crystals, one placed before and one after the sample, serve, by multiple reflection, to produce a narrow incident beam (in our case 12 s wide at half maximum). By this technique, highly collimated incident beams are produced without the necessity of using complicated slit geometries (Fig. 1). Since the rocking angle of these almost perfect crystals is so small (9 arc s) the analyser crystal and counter is placed only a few centimetres from the sample, instead of several metres, which would be required with a conventional low-angle camera. A resolution of $10,000\text{ \AA}$ is attainable, and the diffracted intensity at very low angles is much greater than with the Kratky camera. Because the beam is defined by the fixed reflection geometry, there is no way of increasing the intensity at the high angle end of the pattern; for the system studied here the intensity was at background level above $300\text{ s } 2\theta$, thus limiting the range of the apparatus to angles less than $300\text{ s } 2\theta$.

The zero position of the apparatus was determined by stepping through the main beam in steps of 1 s, with the beam attenuated to within measurable intensity by means of suitably calibrated copper filters. After the zero was located, the filters were removed and the patterns were recorded. Readings were taken between 12 and $300\text{ s } 2\theta$ with $\text{CuK}\alpha$ radiation ($\lambda = 1.54\text{ \AA}$). The sample was contained in a cell with a 1-mm path length with windows made from cleaved mica sheets, and the intensity difference between the cell full and empty after corrections for absorption varied from 30% at the lower end of the angular range angles to 15% at the higher end.

Table 1 Distances corresponding to peak maxima

EB/nucleotide	d_{Bragg}
0 (nicked)	
0 (un-nicked)	2,100
1:20	3,355
1:10	5,250
1:3	3,035

Fig. 2 Diffracted intensity (log scale) plotted against diffraction angle (2θ) in arc s. Curves: 1, Un-nicked PM2 DNA; 2, as 1 with EB added in 1:20 EB/nucleotide ratio; 3, as 1 with 1:10 EB/nucleotide; 4, nicked PM2 DNA; 5, as 1 with 1:3 EB/nucleotide. Inset, plot of a portion of curve 1, with error bars.



The scattering curves, corrected for background, are shown in Figs 2 and 4. We consider first the curves of Fig. 2, which show the scattering at very small angles. The molar concentration ratios of EB nucleotide are listed in the legend. A distinct, well defined peak is evident in curve 1 for supercoiled DNA without added EB. As EB is added, this peak moves in to lower angle, indicating an increasing correlation distance associated with the peak. In curve 3 the second-order peak is also evident, since the primary peak position (corresponding to a distance of $5,250\text{ \AA}$) occurs at such a small angle that the secondary peak is within the detectable range of the instrument.

Curve 4 is for a nicked sample where the superhelix is completely unwound. The scattering curve is smooth; the peak has disappeared. It reappears again in curve 5, at which concentration of EB the completely unwound superhelix begins to coil up in the opposite sense¹². The values of the distances corresponding to the peak maxima are listed in Table 1.

Figure 3 shows the reciprocals of the Bragg distances calculated from the peak maxima as a function of EB concentration. The relationship between the two is reasonably linear, except for the point on the left which is too high. This is in agreement with Freifelder's⁸ observation that the stiffness of the superhelix increases with addition of EB. Thus, Figs 2 and 3 are consistent with and most directly explained by ascribing the source of the scattering to a periodicity of the superhelix itself.

The peaks and the way they vary cannot be ascribed to maxima of a scattering function characterising the size and

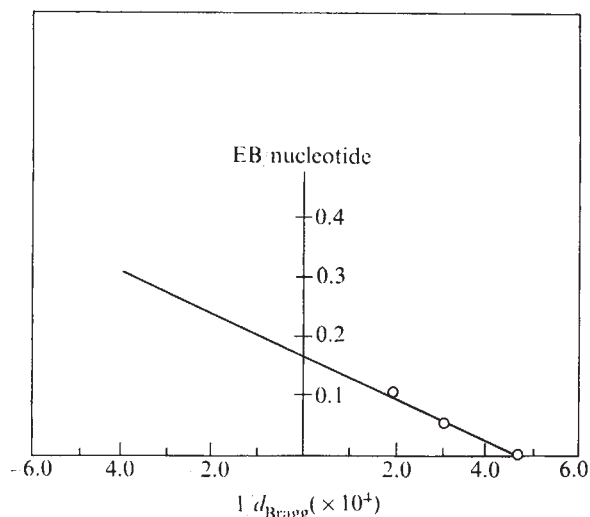
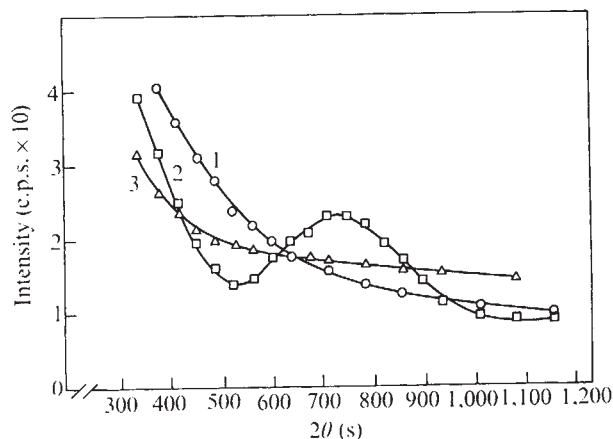


Fig. 3 EB/nucleotide ratio plotted against the reciprocal of the peak spacings; this latter is proportional to the rotation angle of the helix (see discussion in text). The 1:3 point is plotted as negative to show that the winding up is in the opposite sense.

shape of the DNA molecule as a whole. As examples, the scattering functions for loose spherical aggregates or elongated rod-like cylinders or ellipses are not consistent with the measurements. In the first case, a sphere radius derived from the position of the first maximum would result in a sphere volume so large that only a fraction (10^{-4}) of it could be filled by the superhelix volume. The length of the PM2 DNA molecule is 36,000 Å. For cylinders with radii of gyration consistent with the inner slopes of the curves, the peaks would have to appear at angles much further out on the scattering curve. Also, in this case, since the added EB causes the peak to move inward, consistent with an unwinding of the helix, its behaviour is opposite to that to be expected for the increase in asymmetry of an elongating rod-like structure, which would, of course, cause an outward shift of the peak. Finally, a random loop of these dimensions would not give peaks of any measurable intensity. This is evidenced by the nicked sample which does not show these effects at all. If we take the scattering curve of the nicked sample as resulting from a scattering function characteristic of the morphology (see also Fig. 4), the scattering function of which the peaks are a part appears to be superimposed on it. It is not possible at present, however, to isolate the scattering function of the helix from the total curve. A proper separation would require a better knowledge of the morphology of the

Fig. 4 Diffracted intensity measured at larger angles. Curves: 1, PM2 DNA with 1:53 EB/nucleotide; 2, PM2 DNA; 3, calf thymus DNA.



molecule, but even more important, an added difficulty is the nicking effect of the radiation on the helix, which makes quantitative intensities extremely hard to measure. The conformation of superhelical DNA is very sensitive to X rays⁹ and one of our major concerns was whether the material would last long enough in the superhelical form for the experiment to be done. After a 6-h experiment, ultracentrifugal analysis of the specimen indicated that 65% of the material was in the superhelical form whereas almost 100% was when the experiment began. This degradation was tolerable because while it changed the peak intensities relative to the background, it did not shift the peaks bodily and the spacings calculated from the maxima are unaffected. Because of the degradation, the intensities of the different curves of Fig. 2 are not directly comparable with each other and do not measure in any way the relative amount of helical material as a function of added EB. Also, because of the uncertainty in peak-to-background ratios, it was not felt appropriate to slit desmear the curves.

Table 2 Values of radius, helix repeat and contour length

<i>a</i> $d_{\text{obs}} = 337 \text{ \AA}$			
	<i>r</i>	<i>p</i>	<i>c</i>
	85	838	994
	90	608	830
	95	513	787
	100	460	779
	125	357	863
<i>b</i> $d_{\text{obs}} = 2,100 \text{ \AA}$			
	510	8,639	9,214
	550	4,124	5,380
	600	3,097	4,879
	700	2,442	5,030
	800	2,188	3,482

Before analysing these results further, we turn to the measurements with the Kratky in the range between 300 and 1,200 s 2θ , shown in Fig. 4. In these experiments the three systems examined were PM2 superhelical DNA with EB added in the ratio of EB/nucleotide = 0.19, corresponding to that concentration where the curve of Fig. 3 intercepts the ordinate (the unwound helix), PM2 superhelical DNA, and calf thymus DNA, which is not superhelical. It can be seen that whereas curves 1 and 3 show no indication of a maximum, curve 2 for the superhelix does. Its intensity is of the same order of magnitude as those of Fig. 2. We thus identify the peak as arising from the superhelical structure. The Bragg spacing corresponding to the peak maximum is $\sim 337 \text{ Å}$. For helical structures randomly oriented, without long range order, the first maximum observed would be the first layer line¹⁰. The reciprocal space radius of this maximum is related to the helix repeat distance and to the helix radius. Since the first maximum of the first order Bessel function occurs at an argument of 1.84, we have from the relationship $2\pi rR = 1.84$, that R for the first maximum is equal to $1.84/2\pi r$, where r is the helix radius. The theory relates the first maximum to the helical parameters through the relationship

$$\left(\frac{1.23}{d_{\text{obs}}}\right)^2 = \left(\frac{1.84}{2\pi r}\right)^2 + \left(\frac{1}{p}\right)^2 \quad (1)$$

where p is the helix repeat. The factor 1.23 in the numerator on the left hand side is introduced to account for the difference in spacing measured in a single randomly oriented helix from that of a crystalline arrangement of helices¹¹. Similarly, for one turn of a helix the contour length c given by

$$c^2 = 4\pi^2 r^2 + p^2 \quad (2)$$

Our measurements give only d_{obs} and we cannot evaluate r and p separately. We can, however, determine values of p for various values of r and these are listed in Table 2, together with

the contour lengths calculated from equation (2). The minimum value of r from equation (1) is 80.2 Å. The values of p in Table 2 lie in the range between 800 and 300 Å, depending on the radius. Comparative values are 600 Å, deduced from hydrodynamic measurements by Gray *et al.*^{12,13}, and 300 Å, calculated by Wang¹⁴. The values of the radii, consistent with our measurements, are considerably greater than the diameter of the double helix, indicating that the hydrated superhelix is considerably

increasing distance upward from the bottom of the model. No correspondence with the elastic and mechanical moduli of the DNA is claimed for the analogue, nor can it depict visually the intervening space between the two double helices; but it shows that if the superhelix conformation is indeed primarily the result of a torsionally strained primary helix, then it will of necessity be accompanied by a second order helix, and this is what our measurements imply.

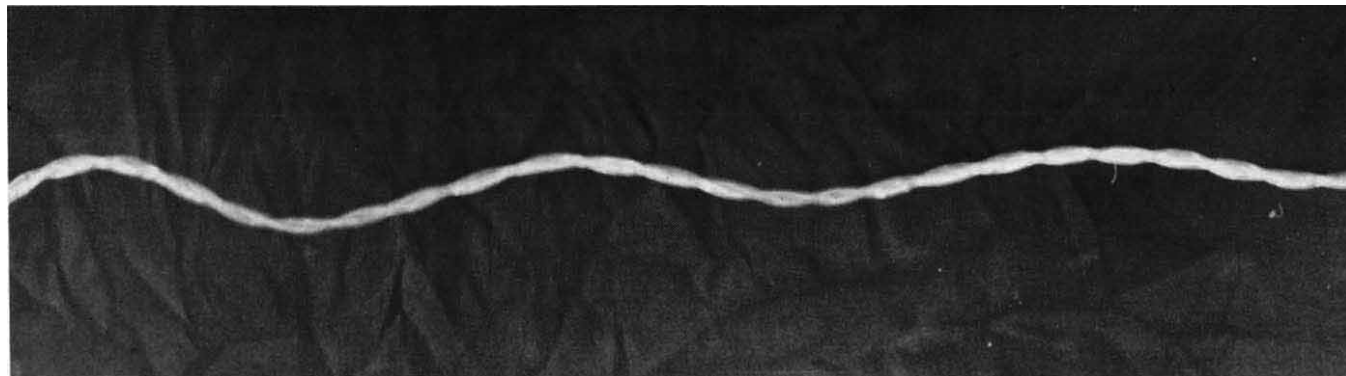


Fig. 5 Photograph of analogue model for the superhelix. The first-order superhelix is represented by the two intertwined chains which run from top to bottom of the model. The second-order superhelix is clearly seen as an additional superimposed helix (see text).

expanded, and should not be regarded as being equivalent to two DNA helical strands tightly wound together. Superhelical formation is the result of residual strain in the double helix, and does not result from attraction between the groups on its exterior; in fact, we would expect a net repulsion between peripheral PO_4^- groups. Thus we suspect that solvent interactions would make an important contribution to the final conformation.

These results place the interpretation of the curves of Fig. 2 on a firmer basis. We believe that they result from scattering from a higher order superhelix which is imposed on the primary superhelix by the mechanical properties of the double helical DNA molecule, whose strain is not completely relieved by the formation of the superhelix. The residual strain is distributed uniformly over the superhelix and leads it to adopt, in turn, a helical conformation of the opposite sense to the superhelix. To illustrate this with a model poses the difficulty of how to approximate the conformation of a strand of DNA when, in addition to being distorted by the residual strain, it is affected by interaction with the solvent medium. The resulting complex local distortions thus produced must be correlated over distances equivalent to the superhelix dimensions, and in general molecular models are not flexible enough to act as useful guides in determining the final conformation of such a complicated molecule when in the liquid state. As a means of circumventing this difficulty and to illustrate how a flexible helical macromolecule under strain is constrained to adopt the configuration we have described, Fig. 5 shows an analogue model. The analogue is made of rubber tubing which is twisted a few times, after which the two ends are joined to form a closed loop. The closed loop is the analogue of the double helical molecule and the imposed twist is the equivalent of the residual strain of the double helical configuration of the DNA. Because of the flexibility of the tubing, the strain is propagated uniformly along its length. When the tubing is released after closing, the loop supercoils. In the photograph the supercoil is identified as the series of small loops which extend from top to bottom of the model. Its period gives rise to the diffraction maxima observed at the higher angles. Its sense is opposite to that imposed on the tubing. Clearly evident in the photograph is another higher order helix of sense opposite to that of the superhelix. The analogue is of course distorted by gravitation, the effect of which can be clearly seen in the photograph, manifested by an increased pitch and decreased radius with

No theoretical analysis of the strain phenomenon exists, so there is no way to estimate how the strain is partitioned between the primary and second order helix, and thus be able to calculate the ratio of the number of turns between the two. We content ourselves with again listing in Table 2b the values of p , c and r corresponding to $d_{\text{obs}} = 2,100$ Å. The minimum radius is ~ 500 Å. For intermediate ratios of pitch to radius, we see from the contour length that the number of superhelix turns in one second order turn is about 6. Furthermore, since the superhelix length is 16,000 Å, there are three second-order turns per molecule.

Because of the nicking effect of the radiation and the low intensities involved, it is not possible to construct a complete composite curve from the two sets of intensity data. The overlap region of the scattering curves is differently affected by the different optical characteristics of the two diffractometers, and as we have noted, it is not possible, because of the nicking, to correct for this. Future work will be directed towards obtaining higher intensities, either by the use of a rotating anode tube, or by incorporation into the DNA of strongly scattering atoms. It will then be possible to extend the angular range of the Bonse-Hart to beyond 1,200 s. The nicking effect will be circumvented by using a position sensitive detector. It will then be possible to apply Fourier transform techniques to the data.

We thank R. Langridge and S. Lapedes for samples of nicked and un-nicked PM2 superhelical DNA and calf thymus DNA, and for stimulating discussions. We also thank D. Jones for help in preparing and standardising some of the samples in which EB was incorporated.

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letters to nature

Phase transition from baryon to quark matter

THE equation of state of matter compressed to supernuclear densities is of considerable interest in astrophysics. For example, pulsars are stars compressed to densities greater than the density of atomic nuclei and are thought to be composed primarily of neutrons, based on detailed calculations of the equation of state of baryonic matter assuming that interaction potentials for the baryons (nucleons and hyperons) are derived from low energy nuclear physics¹. There is, however, considerable evidence from high energy physics that baryons have structure, and it is currently believed that this structure is due to the fact that all hadrons consist of quarks bound by vector gluons². The fact that free, isolated quarks have not been observed has led to the speculation that quarks may be permanently bound inside hadrons³. Since the quarks inside the nucleon behave as free particles², the forces confining the quarks apparently become strong only when the distance between quarks exceeds the radius of a nucleon, $\sim 10^{-13}$ cm. When the density of matter is increased beyond the point that the mean distance between quarks in different baryons is $\ll 10^{-13}$ cm the description of matter as interacting baryons becomes invalid, and instead, a description of matter composed of quarks becomes relevant. At sufficiently high densities matter will behave like a relativistic gas of free quarks⁴ with $P \simeq p/3$, where P is the pressure and p is the energy density of matter. The density at which the baryon to quark transition occurs is of crucial importance to the structure of pulsars. General relativity implies that for a given equation of state there is a maximum energy density for stable stars¹. It is therefore of considerable interest to know whether the energy density of baryon matter at the baryon-quark phase transition is less than this maximum value. Indeed, it has recently been suggested⁵ that the existence of quark matter inside pulsars would allow larger masses for pulsars than was previously thought possible. We will show, however, that there are reasons to doubt this.

To determine the density for the baryon-quark matter transition one needs a theory of quark confinement. Although a detailed theory of confinement is not yet available, a phenomenological theory has recently been proposed⁶ in which the confinement is caused by a universal pressure B on the surface of any region containing quarks. Such a description is consistent with relativity and accounts for many observed

the density at which baryon matter changes to quark matter at zero temperature. At a fixed pressure P and zero temperature the appropriate phase of matter is the one which has the lowest Gibbs energy $\mu = (P + \rho)/n$, where n is the conserved baryon number density. Equating the Gibbs energy for quark matter and for baryon matter at the same pressure then determines the transition point.

Assuming that quark confinement is due to a phenomenological pressure B in the domain occupied by quarks, and that quarks moving inside this domain interact weakly by gluon exchange⁶, it is straightforward to calculate the thermodynamic functions of quark matter. The ground state of quark matter is a Fermi gas with all 'colour' states occupied for each level up to the Fermi level. When the Fermi energy of the quarks is large compared with the quark masses the energy density at zero temperature on dimensional grounds has the form

$$\rho = An^{4/3} + B \quad (1)$$

where A is a constant proportional to $\hbar c$, and n is conserved even when baryons no longer exist. The constant A depends on the dimensionless quark-gluon coupling constant g , and to second order in g we obtain

$$A = \frac{9}{4} \left(\frac{3\pi^2}{\kappa} \right)^{1/3} \left(1 + \frac{8g^2}{3\pi} \right) \hbar c \quad (2)$$

where κ is the number of quark 'flavours' contributing to the energy density. From equation (1) we obtain the pressure P of quark matter

$$P = \frac{A}{3} n^{4/3} - B \quad (3)$$

and correspondingly the Gibbs energy per unit baryon number, μ , as a function of P for zero temperature

$$\mu = 4 \left(\frac{A}{3} \right)^{3/4} (P + B)^{1/4} \quad (4)$$

Table 1 Baryon energy density (ρ_c) in units of $10^{15} \text{ g cm}^{-3}$ where baryons begin to disappear

	$g^2 = 0.55$ $B = 9.5 \times 10^{34}$ erg cm ⁻³	$g^2 = 0.75$ $B = 5.1 \times 10^{34}$ erg cm ⁻³
Pandharipande-Smith (3)	2.7	4.0
Bethe-Johnson (I)	6.5	9.0
Bethe-Johnson (VH)	13	18

The values of g^2 and B are two sets of MIT 'bag' parameters which agree with observed hadron properties⁷, with $\kappa = 3$. Pandharipande-Smith and Bethe-Johnson refer to two recent models of baryon matter^{8,9}.

properties of hadrons when the effect of gluon exchanges between quarks is included⁷. We obtain here the equation of state of quark matter in this theory including gluon effects to second order in the gluon-quark coupling constant g . We then apply Gibbs' criterion for a phase transition to calculate

To find the baryon-quark phase transition we must equate μ as calculated from equation (4) with calculations of μ for baryon matter. Typical results of such a comparison are shown in Table 1. The results are given for the best currently available estimates⁷ of g and B and for two recent calculations^{8,9}, of the properties of cold baryon matter. It can be seen that the transition takes place at baryon energy densities which are 10–60 times the baryon energy density in normal nuclei. Both the quark number density and energy density at the transition are higher than the corresponding baryonic densities indicating that this is a first-order transition. Since the baryon matter calculations were based on nucleon-nucleon potentials derived from low energy nuclear physics their validity at the transition density is open to question. Nevertheless, the calculations should be reliable in their indication that the transition takes

place at densities considerably in excess of those in normal nuclei. Furthermore, since the baryon-quark phase transition occurs at an energy density which is higher than the maximum density calculated for neutron stars^{9,10} speculations⁵ that the existence of quark matter inside pulsars would allow larger masses for these objects than had been calculated using baryon equations of state are unfounded.

Applying the equation of state for quark matter, equations (1-3), we now evaluate the maximum density P_m for stable quark stars in general relativity. This density is given approximately by the condition that $\gamma = \gamma_c$, where $\gamma \equiv d \ln P / d \ln \rho$ is the adiabatic index for quark matter ($= \frac{1}{3}(1 + \rho/P)$ from (3)) and γ_c is a universal function of P/ρ (ref. 11). From this condition we find $\rho_m = 8.3B$ which is lower than the energy density, the baryon-quark phase transition for all the cases investigated here (see Table 1). Thus, a consistent application of the phenomenological MIT model for quark confinement to second order in g leads to the conclusion that stable quark stars at zero temperature do not exist.

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Note added in proof: after submitting our letter we received a preprint by G. Baym and S. Chin in which similar results for the baryon-quark phase transition are obtained.

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High electric fields from industrial stack plumes

THE use of electrostatic precipitators on an industrial stack to remove particulate matter and aerosol particles is now quite common. The plume emanating from such an installation is likely to be highly electrically charged, and consequently associated electrical effects should be observable downwind of the stack.

Measurements were carried out from July 6 to July 10, 1976 of the vertical component of the electric field downwind of a 110-m chimney stack of a cement works at Eastgate, Co. Durham, using a series of conventional field mills¹. Eastgate, ~33 km W of Durham City, is sited in a broad valley, 235 m above m.s.l., running E-W into the Pennine chain. The weather conditions were slightly unstable and cloudless with an easterly wind of between 3 and 4 m s⁻¹ measured at 2 m above ground. Averages of readings taken over 10-min periods at 15-s intervals yield electric field values in the region of +4,000 V m⁻¹ at sites within 500 m of the stack. Peak values up to +10,000 V m⁻¹ were recorded within the same distance from the stack. The direction of the electric field corresponds to a negatively charged plume caused by an applied -60 kV to the corona wires of the electrostatic precipitators. The high electric field values can be compared with a typical value of the normal fair weather electric field upwind of the stack of ~-200 V m⁻¹.

Large perturbations in the normal electric field were observable for distances up to 8 km downwind of the chimney stack. The decay in the electric field, E_P , at a position P was found to

agree with the fundamental line charge equation², modified by an empirical dissipation factor $\exp(-x/d)$ as follows

$$E_P = \frac{\lambda}{2\pi\epsilon_0 h} \left[1 + \frac{x}{(x^2 + h^2)^{1/2}} \right] \exp(-x/d) \quad (1)$$

where λ is the electric charge per unit length, h is the height of the plume, x is the distance of the position P downwind of the stack, ϵ_0 is the permittivity of free space, and d is the distance at which the electric field is reduced to $1/e$ of its initial value. Experimental results indicate that d possessed values of between 1 and 2 km. The effect of turbulent diffusion in the plume was estimated from the plume observations to be sufficiently small to validate the line charge approximation up to at least 2 km downwind of the stack.

In view of the high values of the electric field, a series of experiments was performed to establish the occurrence of point discharge. Measurements of point discharge current from an insulated elevated point, 9.25 m above ground level, were made at 15-s intervals by means of a Keithley electrometer, model 640, 700 m downwind of the stack. Simultaneous measurements were made of the electric field at a distance 10 m upwind of the point. It was found that an electric field of 2.2 kV m⁻¹ was necessary to initiate the point discharge. A maximum value of point discharge current of 3 μ A was observed over a 20-min period. It was also found that the point discharge current, i , and the electric field, E , followed the theoretical relationship³

$$i = k(E - E_0) \quad (2)$$

closely, where k is a constant, E is the value of electric field and E_0 is the onset value of electric field for the occurrence of point discharge.

In studying the dispersal of plumes by atmospheric turbulence, the electrical methods described here may provide a useful supplement to conventional concentration and dosage measurements^{4,5}. The flow of negative charge away from the stack in the form of the charged plume leads to an equal positive current flow down the stack to Earth. Taking into consideration the numerous chimney stacks in operation, it is reasonable to assert that this hitherto unknown charge separation process could significantly modify estimates of the Earth's electrical current 'balance sheet'⁶.

The presence of large electric fields in the vicinity of certain chimney stacks must result in significant point discharge currents occurring from sufficiently high metallic objects. If the objects concerned are poorly earthed they could acquire large electric potentials, which may well lead to electric spark production. This would not be of any practical consequence in most cases. If, however, the atmosphere downwind of the stack contained inflammable material, caused for example by an industrial leak, the occurrence of sparks could result in the ignition or detonation of the material.

The preliminary work described here will be elaborated in more detail in a subsequent publication.

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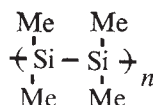
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Heat-resistant Fe–Cr alloy with polycarbosilane as binder

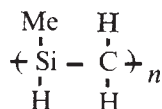
WITH the advances in recent years in high temperature gas-cooled reactors, jet engines and rockets, the constraints on heat-resistant materials are becoming more and more stringent; they must display high temperature strength, thermal shock resistance, high temperature corrosion resistance and toughness. Conventional heat-resistant materials can no longer meet these conditions¹. We report here the development of a new heat-resistant Fe–Cr alloy which has the required properties, based on the method for the conversion of an organic silicon polymer to an inorganic compound by heating which we have described previously^{2–5}.

Polycarbosilane (PC) is prepared as follows: suitable quantities of metallic sodium and xylene are mixed, and then heated to ~110 °C in a stream of inert gas. Dimethyldichlorosilane is then added. The chlorine in the dimethyldichlorosilane reacts with sodium on stirring, producing sodium chloride. By this reaction dimethyldichlorosilane turns into polysilane



which precipitates in the reaction vessel. The substance is recovered by filtration, washed and dried.

The purified polysilane is then put in an autoclave, and heated at 460 °C for 14 h with continuous stirring. The polysilane is thus converted to PC



with an average molecular weight of ~300–800. This is then heated to between 200 and 260 °C *in vacuo*, to remove the light molecular weight fraction by evaporation, and one is left with PC of average molecular weight ~1,000–2,000.

A mixture of powdered Fe–Cr alloy containing 13 per cent by weight of Cr (average grain size 3 µm) and PC of average molecular weight ~1,000 are weighed out in a ratio of 9:1. *n*-Hexane is added, the three are well mixed by kneading and the mixture is dried in air. At this stage, the particles of Fe–Cr alloy are uniformly covered with PC. It is hot pressed to obtain the moulding, using a carbon jig, at a pressure of 19.6 MPa in an atmosphere of Ar, with heating by high frequency induction, capable of giving 300 °C h⁻¹ and the moulding is kept at a maximum temperature of 1,100 °C for 30 min. The pressure is then released, the high frequency power source is switched off, and the moulding is left to cool naturally.

Powder X-ray diffraction using an Fe target with a Mn filter and transmission electron microscopic observation indicate the formation of spherical or ellipsoidal particle of Cr₇C₃ and CrSi₂ with a diameter of 1,500–2,500 Å. When polycarbosilane alone is heated at 1,000 °C in an inert gas

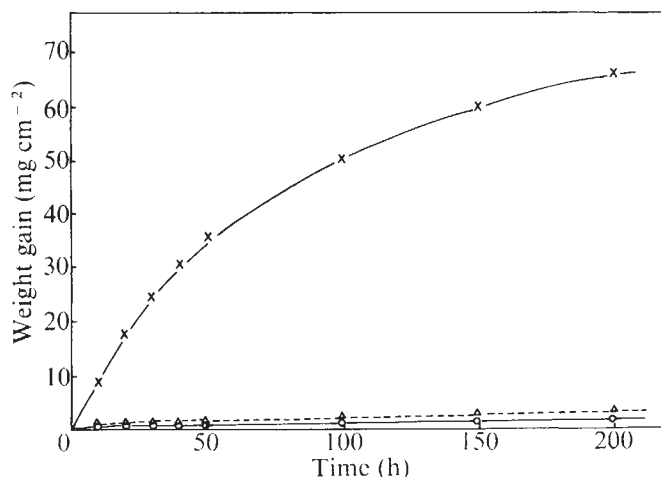


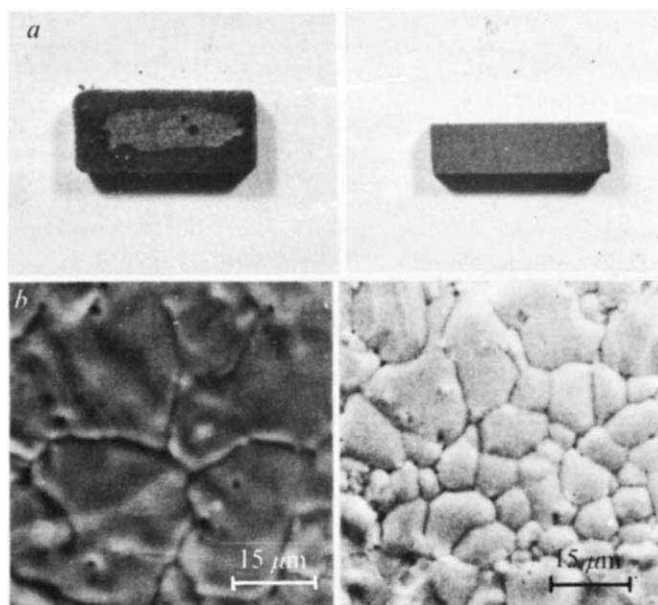
Fig. 1 The gain in weight against the heating time in air at 1,000 °C for the Fe–Cr alloy (x) and the Fe–Cr 10% by weight PC alloy (O); Δ, Ni–22Cr reference.

atmosphere, it decomposes, releasing CH₄ and H₂. The result is a skeleton of SiC, which is amorphous. If the Fe–Cr particles coated uniformly with PC are cold pressed and then heated at 1,100 °C, a reaction occurs between the Fe and Cr in the matrix and the amorphous SiC dispersed uniformly through the alloy, resulting in the formation of the silicide and carbide. A uniform distribution of silicide and carbide in the alloy is not obtained by introducing commercial α- or β-SiC into the powdered alloy and hot pressing.

Oxidation tests were made on the moulding obtained above, on a moulding made without PC and on the well known heat-resistant Ni–22Cr alloy as a reference, by heating in an electric furnace at 1,000 °C for 50 h. Figure 1 shows the weight gain from oxidation against the heating time for the Fe–Cr alloys at 1,000 °C in air. The weight increases in a parabolic curve in the Fe–Cr alloy without PC, but hardly increases at all in the moulding made with PC.

The surfaces of the mouldings after the tests were examined (Fig. 2a). Spalling of the oxidation film was found in the moulding without PC, but in the moulding with PC,

Fig. 2 a, Oxidation film of the alloy with (right) and without (left) PC after exposure to air for 50 h at 1,000 °C. b, Scanning electron microscopic observation of grain size in the two alloys (right with PC, left without).



only a slight film was observed which was firmly attached to the surface. This high resistance to oxidation may result from the stability of Cr_7C_3 and CrSi_2 against oxidation.

A value of 345 for the Vickers microhardness was obtained for the PC-coated alloy at room temperature—it is 100 in the PC-less alloy. Abrasion tests were made on the Fe–Cr alloys with Okoshi's testing apparatus. A disk of thickness 3 mm and diameter 30 mm was placed on the square $40 \times 40 \times 10$ -mm specimen, and a load of 7 kg was placed on the disk. The disk was rotated at 67 rad s^{-1} at room temperature. The value obtained for the abrasion resistance was $1.48 \times 10^{-6} \text{ mm}^2 \text{ kg}^{-1}$ in the Fe–Cr alloy with PC, and $7.10 \times 10^{-6} \text{ mm}^2 \text{ kg}^{-1}$ in the same alloy without PC.

The grain size of the mouldings heated at $1,000^\circ\text{C}$ for 5 h was examined with a scanning electron microscope after etching the surface with aqua-regia dissolved in glycerine. As seen in Fig. 2b, the grain size is $25 \mu\text{m}$ in the Fe–Cr alloy without PC, but in the alloy with 10% by weight of PC it is $9 \mu\text{m}$.

The large Vickers microhardness and the excellent abrasion resistance of the Fe–Cr alloy with PC can possibly be attributed to the existence of small particle Cr_7C_3 and CrSi_2 and the small grain size. This alloy may find many applications where heat resistance plus abrasion and oxidation resistance is required.

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SiC sintered bodies with three-dimensional polycarbosilane as binder

As is well known, silicon carbide is a practical heat-resisting material, because it has high strength and excellent oxidation, corrosion and thermal shock resistance. It is, however, difficult to obtain it as a sintered body because of its poor sintering characteristics. The usual method¹ for obtaining SiC with high flexural strength are to add a sintering promoter to fine SiC powder followed by hot pressing. The process is complicated and not suitable for obtaining large products. We here report the synthesis of high purity SiC mouldings with high mechanical strength by low temperature ($1,000$ – $1,400^\circ\text{C}$) sintering of SiC powders bond with three-dimensional polycarbosilanes. The method is based on the conversion of an organosilicon polymer to the inorganic compound by heat treatment^{2–5}.

The SiC powders used are 99.99% pure α -SiC, with an average particle size of $3 \mu\text{m}$. To these are added 10% by weight of polycarbosilane (PC)^{6,7} with an average molecular weight of 800 dissolved in a suitable quantity of *n*-hexane, which is then removed by evaporation. Particles $10 \times 30 \times 4$ mm in size were obtained by pressing at 196 MPa at room temperature. Their temperature was raised at 100°C h^{-1} to 700 – $1,400^\circ\text{C}$ in a flow of 200 ml min^{-1} of N_2 , and kept at the desired temperatures for 1 h. Sintered SiC particles were produced; there were no shrinkage or expansion.

The flexural strength of the SiC at room temperature was measured in three-point bending test with an Instron

bending tester under ASTM testing conditions. The $10 \times 30 \times 4$ -mm specimen was tested at strain rate $8.3 \times 10^{-3} \text{ s}^{-1}$ with the span of 20 mm. Figure 1 shows the bending strength against the temperature at which sintering occurred. The bending strength increases with temperature up to $1,100^\circ\text{C}$, beyond which it keeps constant at 61.7 MN m^{-2} .

To increase the strength still further, 'PC impregnation' was carried out. SiC particles obtained as above were heated together with PC powder in an autoclave under a vacuum at 100°C for 30 min, and then heated at 350°C for 1 h at a pressure of 9.8 MPa (of Ar). The particles were

Table 1 Characteristics of SiC with 10% by weight of polycarbosilane and also KT-SiC

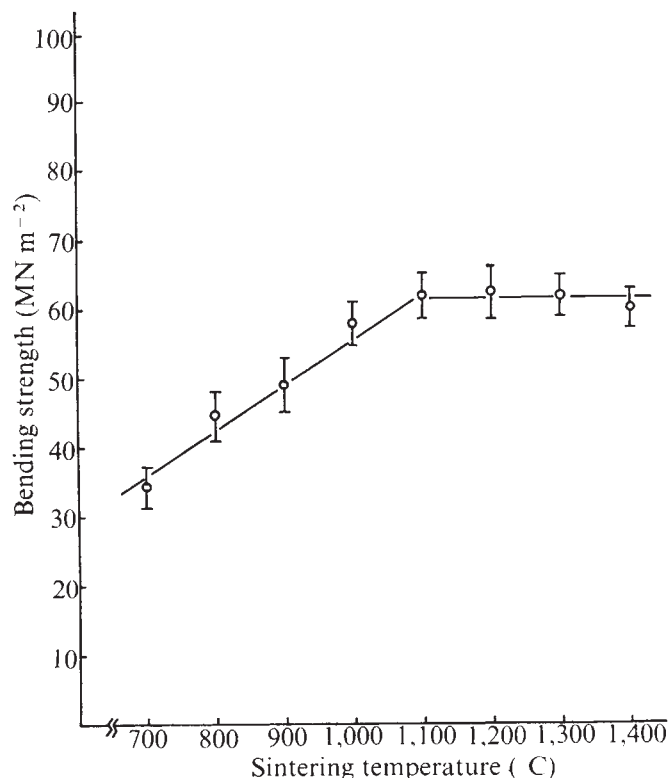
	SiC with PC	KT SiC ¹
Production temperature ($^\circ\text{C}$)	1,400	2,300 (hot press)
Impurity	$\sim 0\%$	Excess silicon or carbon
Apparent density (%)	70–80	100
Bending strength (MN m^{-2})	250	~ 168
Oxidation/acid resistance	Very high	Very high
Formability and dimensional precision	Excellent	Poor

then heated up to 700 – $1,400^\circ\text{C}$ at 100°C h^{-1} under a flow of N_2 . They were kept at these temperatures for 1 h.

The effect of PC impregnation is indicated in Fig. 2. It is seen that the apparent density and the bending strength both increase linearly with repetition of the process. The apparent density of the SiC particles after five cycles is 83.0% and the corresponding bending strength is 250 MN m^{-2} . A feature of the SiC particles thus fabricated is their exceptionally high bending strength for a low density. In Table 1, one sees that the bending strength of our SiC is 250 MN m^{-2} , while it is $\sim 168 \text{ MN m}^{-2}$ for KT-SiC.

The amount of PC added to the SiC was varied—and the addition of 10% by weight was found to be the most suitable. For a sintering temperature of $1,000^\circ\text{C}$ for example,

Fig. 1 Bending strength against sintering temperature.



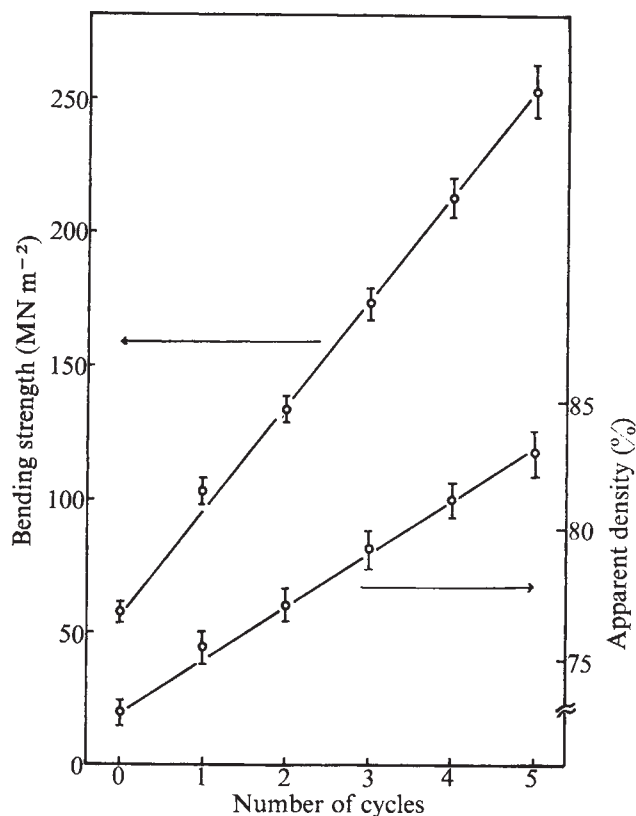


Fig. 2 Bending strength and apparent density against repetition of the PC impregnation cycle; sintering temperature 1,000 °C under flow of N₂, without heating in the air.

the bending strengths were 24.5 MN m⁻² for 5% by weight, 61.7 MN m⁻² for 10% by weight and 24.5 MN m⁻² for 15% by weight.

That SiC with PC has high mechanical strength, is interesting for the theory of sintering. In another experiment, SiC powder (particle size ~3 μm) and very fine SiC particles (~100 Å) (refs 2, 3) obtained by the decomposition of PC were sintered by heating. No contraction or expansion was observed. The diffusion coefficient of C in SiC is small— 3×10^{-13} cm² s⁻¹, at 1,800 °C, (S. Prochazka, unpublished)—so the mechanism cannot be simply explained by the normal idea of diffusional sintering. There is no dimensional change, and therefore one possibility may be condensation by PC evaporation.

When SiC particles were kept at 1,400 °C in air for 2, 5 and 10 h, the gains in weight from oxidation were 4.41, 5.70 and 7.01 mg cm⁻², respectively. Beyond 10 h, there is no increase. Bending strength after oxidation was measured. For sintering at 1,000 °C under N₂ flow, for example, it was 56.8 MN m⁻² in Fig. 1, but increased to 147 MN m⁻² after being kept in air at 1,400 °C for 10 h—the SiC is not only resistant to oxidation, but its bending strength increases. This phenomenon is being studied at present, but preliminary powder X-ray diffraction indicates that a very small amount of Si₃N₄O was produced on oxidation and this acts as a binder, thereby raising the bending strength.

The SiC was immersed in boiling solutions of 20% HCl and 50% HNO₃ for 100 h. It would have lost 0.05 mm of surface material in a year, and is therefore extremely stable in boiling acid solutions.

For SiC in current use, a different substance is used as the binder, so the SiC content in them cannot be >98%. For SiC with PC, however, there is hardly any chance for other elements than Si and C to be present. The product is thus of high purity; chemical analysis showed the SiC content to be almost 100%.

The SiC thus developed are easily produced industrially and may find a use in many fields, including the construction of chemical plants.

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Solar activity geomagnetic field and terrestrial weather

It has long been known that geomagnetic disturbances tend to recur after ~ 27 d (refs 1–4). More recently, this effect has been associated with the rotation of the interplanetary magnetic field^{5–8}, and some evidence for a relation between the interplanetary magnetic field and terrestrial weather has also been found^{9,10}. The interplanetary magnetic field is usually characterised by either two or four sectors, and this pattern rotates with a fairly well defined period of $\sim 27.1 \pm 0.1$ d (ref. 7). Such a periodicity leads naturally to the use of spectral analysis as an independent test of the reported association between the interplanetary magnetic field structure and terrestrial weather. We have obtained data on the geomagnetic activity index A_p for the 1964–70 from the National Oceanic and Atmospheric Administration and the vorticity area index used by Wilcox and his colleagues in their analysis⁹ for the same interval. We wish to form spectra of both time series and examine the spectra for common features which may be associated with solar related phenomena. Specifically we look for peaks in the power spectra of both time series with periods near 27.1 d.

Both the A_p and vorticity area index exhibit annual variations and long term trends. The annual variations and long term trends were removed from both data sets and the resultant series normalised so that the average of each index is zero. We have calculated least-squares estimates of the Fourier components of both normalised data sets for 1,921 periods (P) between 4 and 64 d. The estimates of the Fourier components a and b were calculated by varying a and b to minimise

$$V_3 = \sum_{n=1}^N \left[x_n - a \cos\left(\frac{2\pi}{P}n\right) - b \sin\left(\frac{2\pi}{P}n\right) \right]^2 \quad (1)$$

where the x_n are the normalised data points and N is the total number of data points.

After we had computed the Fourier components for both indices, we needed some way to compare the spectra and assess the significance of the results. We also needed some check that any periodicity found was indeed solar related, since both indices are of terrestrial origin. We have compared the spectra in three different ways.

One common method of assessing the significance of a least-squares fit¹¹ is to compare the residual deviation V_3 to the deviation of the data before fitting

$$V_1 = \sum_{n=1}^N x_n^2 \quad (2)$$

by forming the statistic

$$F_{2,N-3} = \frac{N-3}{2} \frac{V_1 - V_3}{V_3} \quad (3)$$

We have calculated the F statistic for each spectrum but, to compare the spectra, we tabulated the lesser of the two F statistics as a function of period. The largest peak in the resultant 'comparison spectrum' occurred at a period of 27.49 d. We may use the F statistic to determine the probabilities that the calculated reductions in variance are due to chance. Following Abramowitz and Stegun¹², we find the probability that the fit at 27.49 d arisen by chance for the A_p spectrum is $\sim 1.2 \times 10^{-4}$ and for the vorticity spectrum is $\sim 1.2 \times 10^{-5}$.

The F statistic calculated from the data is distributed only approximately as $F_{2,N-3}$, so that using such low probability estimates is probably not justified. We therefore arbitrarily use the lowest probability tabulated by Abramowitz and Stegun¹³ for the F distribution which is 10^{-3} . If the A_p and vorticity indices are independent, then the probability that both indices would exhibit fits of this significance for a given period is $\sim 10^{-6}$. To obtain a significance estimate, it is essential to allow for the fact that the period was not chosen *a priori*, but inferred from the data. A generous estimate of the range of periods to be investigated is the range of observed synodic rotation periods of the photosphere of the Sun, namely 26.87–28.98 d (ref. 14). Accordingly, least-squares fits were calculated for 22 periods in this range. The probability of obtaining at least one of 22 probability estimates as small as 10^{-6} is $\lesssim 2.2 \times 10^{-5}$ if all of the periods are statistically independent. The length of our sample implies an intrinsic line width of $2.46 \times 10^{-3} \text{ d}^{-1}$ and the spacing between the 22 periods is $0.77 \times 10^{-3} \text{ d}^{-1}$. This indicates that not all of the 22 periods can be considered independent. We therefore think that calculating the probability estimates as if the 22 periods were independent produces a conservative probability estimate. Since for reasons stated above, we apply an arbitrary cutoff at 10^{-3} to estimates based on the F statistic, we have also calculated an empirical probability estimate. The F statistics for all the 1,921 periods analysed were ranked, and the probability of each fit estimated by the rank of the fit divided by the number of periods for which each spectrum was estimated. To compare the spectra, the larger of these two probabilities was tabulated as a function of period. There were two periods (19.88 and 44.52 d) with (chance) probabilities as low as or lower than the probability for the 27.49-d period. All three probabilities were, however, close together (26/1921, 27/1921, and 27/1921) and the next most significant peak had a probability estimate approximately twice as large (52/1921). If we combine the two empirical probability estimates in the same manner as the estimates from the F test, we find that the peak at 27.49 d has the smallest probability estimate ($\sim 3 \times 10^{-5}$) of any of the periods analysed by more than a factor of two. When we compensate for the fact that the period is chosen *a posteriori*, the empirical probability estimate that the observed result was produced by chance becomes $\lesssim 7 \times 10^{-4}$. Since both the A_p and vorticity indices are terrestrial, the assumption that the two indices are independent may not be justified. We therefore

explored a third procedure, based on the assumption that the relation between the A_p index and the interplanetary magnetic field structure is sufficiently well established that we may use the A_p spectrum to establish a rotation period. This assumption implicitly involves the supposition that there are no periodic meteorological phenomena with periods close to the solar rotation period that affect the geomagnetic activity index. We may then accept the probability estimates for the 27.49-d period in the vorticity spectrum. These estimates are $\lesssim 10^{-3}$ and $\sim 1.4 \times 10^{-2}$ for the F test and empirical probability estimates respectively.

Even if we accept the reality of the peaks in the spectra at 27.49 d, it is desirable to have some check that these peaks are associated with solar rotation. One fairly simple check is based on the fact that the Earth's orbit is slightly eccentric, and this eccentricity causes a variation of the apparent rotation period of the Sun through the calendar year¹⁵. The eccentricity of the Earth's orbit may easily be taken into account (to first order in the eccentricity) by slightly modifying the argument of the sine and cosine functions in equation (1). We have generated 'eccentricity corrected' spectra for both data sets and evaluated the results in the same manner as the uncorrected spectra. The F statistics for both A_p and vorticity indices were higher in the 'corrected spectra', but the probability estimates from the F test were not changed since we adopted an arbitrary cutoff at 10^{-3} . For the empirical probability estimates, the results were approximately the same as those for the uncorrected spectra; however, other peaks were degraded, with the result that the peak at 27.49 d had the lowest rather than the second lowest probability estimate. If we accept the period determined from A_p spectrum as a solar rotation period, the empirical estimate of the probability that the calculated fit in the vorticity index 'corrected' spectrum was produced by chance is $\lesssim 1.3 \times 10^{-2}$.

We have attempted to assess the significance of the observed peaks under various assumptions. The results vary from $> 99.99\%$ to $\sim 98\%$. We feel that the former estimate is unrealistically high; however, the decision to accept any of the estimates is subjective. Our own feeling is that the most reasonable procedure is to accept the period derived from the A_p spectrum as solar related. We therefore feel that the results of our analysis are significant at the 98% level. We do not feel that our analysis establishes a connection between the vorticity area index and solar activity, but we do feel that it provides supporting evidence for such a connection.

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Volatilisation from solid particles of the regolith

WE have previously¹ dwelt on the possible volatilisation of some elements from continuous melts of lunar surface material as a result of which it becomes depleted in elements such as Na, K and Rb, and so on. The mechanism of volatilisation proper was not confined to the liquid phase, and so they can also volatilise from the solid phase. Moreover, if solidification is not accompanied by crystallisation, the temperature dependence of the volatilisation time constant τ_v found in ref. 1 should remain unchanged.

Table 1 Calculated values τ_v for volatilisation of Na from lunar material (terrestrial time)

Particle size (cm)	Surface temperature (K)			
	1,500	900	500	300
1	15 h	5 d	25 d	300 yr
10^{-2}	0.15 h	1.2 h	0.25 h	3 yr

Table 1 gives the calculated values τ_v for the volatilisation of Na (see ref. 1) from the liquid and solid lunar material. For regolith particles the value τ_v is negligible compared with the geological time scale since $\tau_v \propto d$.

In ref. 1 the assumption that the lunar surface material was liquid was used only so that the complicated process of the transport of volatile elements to the surface could be ignored. In the solid phase, however, it is just their transport to the surface that may be the weak point of the calculation of the depletion process.

In this case the transport mechanism is mainly diffusive and, hence, a simple estimate of the diffusion time constant τ_d can be carried out

$$\tau_d = \frac{d^2}{D}; \quad D \sim \exp(-E_a/RT_s)$$

where D is the diffusion coefficient and E_a is the diffusion activation energy.

Since the dependence of τ_v on d is linear¹

$$\tau_v = \tau_{0v}d \quad (1)$$

where

$$\tau_{0v} \sim \exp[-(L-A)/RT_s]$$

and $(L-A)$ is the effective heat of evaporation^{1,2}, the depletion process will be limited by volatilisation ($\tau_v \gg \tau_d$) in the region of the smallest particles and by diffusion ($\tau_d \gg \tau_v$) in the region of large particles. The boundary between these regions $d = d_1$ can be determined from the condition $\tau_v = \tau_d$; then

$$d_1 = D\tau_{0v} \sim \exp[-(E_a - (L-A))/RT_s] \quad (1a)$$

The quantities E_a and $(L-A)$ have a similar physical meaning, and the difference between them $E_s = E_a - L + A$ should be close to the heat of adsorption, that is, it should be of the order of kcalorie mol⁻¹ (ref. 2).

Using equation (1), one can show that, at all temperatures of interest ($250 \text{ K} \lesssim T_0 \lesssim 1,500 \text{ K}$), the value of d_1 lies within the region of regolith particle sizes, $\sim 10^{-4}$ – 10^{-1} cm, and because $E_s > 0$ it increases with decreasing temperature.

In general, putting $\tau_0 = \max(\tau_v, \tau_d)$, one may assume that the rate of depletion of volatile elements from lunar material is determined by the time constant of the process 'weak point' τ_0 ($\tau_0 \lesssim 10^4$ yr for $d \lesssim 10^{-1}$ cm and $250 \text{ K} \lesssim T_s \lesssim 400 \text{ K}$). This interval corresponds approximately to the temperature

variations, T_s , of the upper layers of the regolith during the lunar day.

Since volatilisation and diffusion occur only in the day, and are almost totally switched off at night, the effective value τ_0 for the lunar day does not increase more than twice.

Next, it should be kept in mind that under meteoroid bombardment, and some other factors, the regolith particles, at least those from the uppermost layer ($h \approx 10^{-1}$ cm), plunge into the lower ($h \lesssim 1$ cm) layers and then rise to the surface, intermixing at the rate $U_p \approx 1 \text{ cm}/10^6 \text{ yr}$ (ref. 3). On the other hand, it is known^{3,4} that the rate of regolith formation is $U_f \approx 10^{-1} \text{ cm}/10^6 \text{ yr}$.

Thus, for the regolith upper layer ($h \approx 10^{-1}$ cm) one has the following inequality

$$\tau_0 \ll \tau_p \ll \tau_f \quad (2)$$

where $\tau_0 \lesssim 10^4$ yr, $\tau_p = h/U_p \approx 10^5$ yr, and $\tau_f = h/U_f \approx 10^6$ yr, τ_p and τ_f being the time constants of the corresponding processes.

The relationship between τ_p and τ_f shows that each regolith particle of a given layer was on the surface > 10 times whereas the first inequality in equation (2) implies that even a single sojourn of a particle on the surface is sufficient for the particle material to be significantly depleted in volatile elements.

Earlier we have shown theoretically and experimentally^{1,5} that the process of volatilisation from the liquid phase as a result of meteoroid impacts is inessential because the volume (size) of each melt is small, and the melts solidify very rapidly. These two factors also rule out the chemical fractionation of the lunar regolith by impact melting, proposed in ref. 6.

Meteoroid bombardment, however, can have a decisive effect on the process of volatilisation of Na, K and other elements from solid particles in the regolith, crushing and intermixing them. If this process really occurred (and occurs at present) on the Moon, then a chemical analysis of lunar rocks of the appropriate sizes could reveal the presence of a volatile-element concentration gradient.

Finally it should be noted that the approach we suggest may be also useful for solving some terrestrial ecological problems like that in ref. 7.

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Concentrations of dissolved copper in the eastern Atlantic Ocean 23°N to 47°N

CONSIDERABLE uncertainties still exist as to the abundances of most of the trace metals in seawater, including some of the more intensively investigated elements. Recent analyses of copper in surface waters south of New Zealand¹ and in waters from various depths over the East Pacific Rise² have given values of 0.06 – $0.40 \mu\text{g l}^{-1}$, and a similar range has been found by M. J. McCartney (personal communication) for samples from the Mediterranean Sea and the north-eastern Atlantic Ocean. A geometric mean concentration of $0.26 \mu\text{g l}^{-1}$ has been reported for samples collected off the shelf west of Scotland³. These values are either below

or at the lower end of the ranges reported for seawater by other workers. For example, extensive measurements⁴ of dissolved copper in surface waters have given average concentrations for various near-shore and open ocean regions which vary between 0.3 and $1.7 \mu\text{g l}^{-1}$, with a mean of $0.8 \mu\text{g l}^{-1}$, and a number of other recent investigations⁵⁻⁸ of North Atlantic Ocean waters also support an abundance value of $\sim 1 \mu\text{g l}^{-1}$. These contrasting findings may reflect real environmental variability but the alternative explanation that such variations are, at least in part, attributable to analytical problems, and especially to contamination, must also be considered. This communication reports values for dissolved copper in samples taken from the eastern Atlantic Ocean in October and November, 1975, which support findings of concentrations of $\sim 0.2 \mu\text{g l}^{-1}$. The results of measurements on these samples of the organically associated copper are summarised.

Surface samples were collected from RRS Discovery from a position forward of any discharges from the moving ship, using a plastic bucket with no metallic parts. Subsurface samples were collected using plastic water bottles. Metallic surfaces on the outside of these bottles, and the messengers employed, were coated with a plastic film. Samples collected from depths of < 500 m were filtered through Sartorius membrane filters of $0.45 \mu\text{m}$ average pore diameter, the filters having previously been leached with HCl. Samples from greater depths were not filtered since only a negligible amount of copper could be contributed from the small amounts of particulate material present. Dissolved copper was concentrated by uptake on Chelex-100 chelating ion-exchange resin after acidification of the sample (~ 800 ml) to pH 5, and the resin was eluted with 2 N nitric acid. The method was basically similar to that of Riley and Taylor⁹. This procedure leads to a measurement of the fraction of the dissolved copper which is available to the chelating resin. For the surface samples and a number of the subsurface samples from depths up to 1,000 m, analyses were also made on aliquots which had been photo-oxidised by ultraviolet irradiation for 5 h, using a 1,000-W medium pressure mercury arc lamp. The values obtained for the oxidised samples represent the total dissolved copper whereas the aliquots not treated in this way may contain some copper which is organically complexed in forms which are unavailable with this method of concentration. Further aliquots of most of these samples, also of ~ 800 ml, were extracted with chloroform at natural pH, without the addition of a complexing agent. The chloroform extracts were evaporated;

the residues were oxidised by evaporation with perchloric acid and redissolved in dilute nitric acid. Blank determinations were made for each procedure using only the reagents.

Concentration was commenced shortly after sample collection; the concentrates in dilute nitric acid were returned to the shore laboratory for determination of copper by atomic absorption spectrophotometry. Evaluation of the method has shown that the coefficient of variation of the procedure, as determined by analysing replicate samples at the level of $0.7 \mu\text{g l}^{-1}$, is $\sim 4\%$; it gives essentially complete recovery of inorganic copper and the results agree closely with those obtained by solvent extraction of the complex formed with ammonium pyrrolidinedithiocarbamate. Blank values were $0.02 \mu\text{g}$ for the concentration procedure and $< 0.01 \mu\text{g}$ for the chloroform extraction.

The difference between the total and available dissolved copper fractions averaged $< 0.02 \mu\text{g l}^{-1}$ and so only the latter values are given in Table 1 with other chemical and hydrographic measurements; the samples were collected in sequence from north to south. The chloroform extractable fractions mostly ranged from undetectable to $0.02 \mu\text{g Cu l}^{-1}$ with a single higher value of $0.04 \mu\text{g l}^{-1}$. In coastal and estuarine waters, organically associated fractions of copper, operationally defined in ways similar to those used here, have been reported to be significant, ranging up to 50% or more of the total copper¹⁰⁻¹³. Our data indicate that in open ocean surface and intermediate waters, the fraction made available by oxidation of organic matter, and that which is chloroform extractable, each account for only $\sim 10\%$, or less, of the total copper. Measurements of operationally defined fractions do not enable any conclusions to be drawn as to the total extent of organic complexation. The difference in the magnitude of these fractions between coastal and oceanic waters, however, may reflect the different sources of organic complexing material in the near-shore and open ocean environments. The lower concentrations of such fractions in ocean waters, as found in this work, will require more sensitive analytical approaches for their fuller elucidation.

The concentrations of available dissolved copper ranged from 0.09 to $0.23 (\text{mean } 0.16) \mu\text{g l}^{-1}$. This range agrees well with some other recent findings as discussed above but is substantially lower than the ranges reported hitherto for the North Atlantic Ocean⁴⁻⁸. For example, concentrations at various positions and depths in the eastern tropical region were found⁷ to range from 0.4 to $12.3 (\text{mean } 1.0) \mu\text{g l}^{-1}$, while for a section from England to Barbados, extending

Table 1 Results for dissolved copper and hydrographic data

Position °N °W	Depth (m)	Salinity (‰)	Temperature (°C)	Dissolved copper ($\mu\text{g l}^{-1}$)	Dissolved silicon ($\mu\text{g atom l}^{-1}$)	Dissolved oxygen (ml l^{-1})
46°31' 09°36'	surface	35.575	—	0.22	—	—
44°52' 10°02'	surface	35.680	—	0.23	—	—
39°06' 10°32'	surface	36.013	18.3	0.22	—	—
38°05' 11°02'	surface	36.018	17.75	0.20	—	—
34°20' 12°40'	surface	—	21.7	0.18	—	—
31°11' 14°04'	surface	—	—	0.18	—	—
25°05' 16°24'	10	36.660	21.80	0.11	0.62	5.17
(Discovery Station 8932; bottom depth 2,560 m)	50	36.630	21.47	0.14	0.48	4.86
	100	36.588	19.12	0.11	0.62	5.92
	203	36.359	16.35	0.20	2.14	4.53
	353	35.946	13.89	0.13	4.69	3.96
	498	35.573	11.56	0.13	8.67	3.32
23°06' 17°51'	10	36.707	21.92	0.13	0.38	5.09
(Discovery Station 8933; bottom depth 2,985 m)	100	36.632	18.08	0.09	1.21	4.86
	431	35.636	11.94	0.10	7.74	3.70
	938	35.143	7.47	0.18	19.6	3.08
	990	35.190	7.04	0.12	19.9	3.34
	1,007	35.152	6.97	0.13	19.6	3.41
	1,513	35.177	5.50	0.23	22.1	4.15
	2,000	—	—	0.17	—	—
	2,176	35.034	3.78	0.21	28.3	5.16

into the eastern Caribbean Sea⁸, surface concentrations of 0.47–1.8 (mean 0.93) $\mu\text{g l}^{-1}$ were found.

Our values for surface waters show a general decrease from north to south over the latitudes concerned. Variations in concentration with depth were not pronounced but suggest a slight depletion in the euphotic zone relative to the deeper water. A comparison of the trends in the concentrations of dissolved silicon and copper in the vertical profiles (Table 1) provides, however, no evidence that transference into deeper water by biological processes is a major influence on the distribution of dissolved copper.

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'Little Ice Age' palaeotemperatures from high altitude tree growth in S. Norway

THE sensitivity of tree growth to climate has led to reconstruction of past climates from long tree-growth records^{1,2}. Growth at the treeline in mountain regions and at the polar limits of tree growth is particularly useful because, in these locations, moisture is normally adequate and growth has been shown to be highly dependent on summer temperatures^{3,4}. Some other environmental factors have been recognised as influences on the growth of trees^{5,6}, but such factors are of relatively minor importance in this type of habitat. I reconstruct here a continuous record of summer temperature fluctuations representative of treeline conditions in the central southern Norwegian fjell since AD 1700. Combination of tree-ring data with information from glacier fluctuations over the same period shows short term oscillations of amplitude 1.0–3.0 °C superimposed on a long term increase in temperature of ~1.0 °C.

Two major limitations appear to restrict the use of tree-growth variations as palaeothermometers. First, even at the treeline, it is rare to be able to demonstrate a sufficiently high dependence of tree growth on summer temperatures. Secondly, calculation of standardised tree-growth indices (necessary to remove the effect of tree age on growth increment) results in the elimination of longer-term trends from the tree-growth record and hence the loss of information about longer-term temperature fluctuations. I show here that summer temperatures which are not subject to the above limitations can be reconstructed from tree growth. A Scots pine (*Pinus sylvestris*) tree-growth record, previously published by Slåstad⁷ from the treeline in upper Gudbrandsdalen (Fig. 1), and shown by him to be highly correlated with summer temperatures from Dombås meteorological station, is analysed for its palaeotemperature implications; independent glaciological and geomorphological evidence from the Storbreen glacier, Jotunheimen, is used to

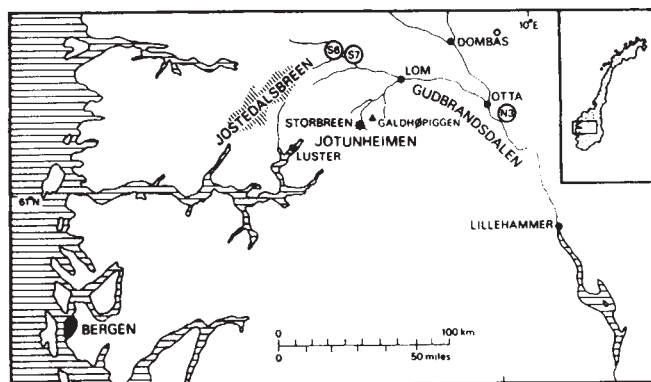


Fig. 1 Location of Slåstad's tree-growth sites—Skjåk 6 (S6), Skjåk 7 (S7) and Nord-Fron 3 (N3)—the Storbreen glacier and Dombås meteorological station.

adjust the record to take into account the long-term change in climate over the period of interest. Slåstad's tree-growth data are shown in unaltered form and after smoothing by harmonic analysis⁸ in Fig. 2.

Based on mean monthly 2 p.m. temperatures from Dombås, Slåstad found the strongest relationship to be between tree growth and an index of June and July temperatures, with July weighted twice, that is $(1 \times \text{June} + 2 \times \text{July})/3$. This relationship has been checked and is illustrated and given in mathematical form in Fig. 3 for the 50-yr period 1901–50 and for two 25-yr subperiods. The correlation coefficient (r) for 1901–50 is $+0.77$ ($p=0.001$); for 1901–25, $r = +0.84$ ($p = 0.001$); for 1926–51, $r = +0.79$ ($p = 0.001$). This relationship between tree growth and summer temperature is considered sufficiently strong to use regression of summer temperature index on tree-growth as part of the basis for reconstruction of temperatures before AD 1900. Before the relationship can be extrapolated into the past any long-term trend in tree growth and climate must be obtained from independent evidence.

Liestøl, working on the Storbreen glacier, has calculated the mass balance of the glacier since AD 1816 (ref. 9) and working on the glacier-foreland, I have reconstructed former glacier margin positions back to AD 1750. This information has been used to estimate the tree growth corresponding to the glacier in equilibrium (that is, no net change in the glacier mass budget and hence no net movement of the glacier margin) from AD 1700 to 1950. The resulting line corresponding to the equilibrium condition of the glacier (EE') is shown in Fig. 2b but full details of the methods used are given by J. A. Matthews, unpublished. Tree growth of less than the indicated critical value corresponds to a glacier advance; glacier retreat is indicated when tree growth exceeds the critical value. Fig. 2b shows that Storbreen was in equilibrium at a lower tree-growth value in the recent past than in earlier times.

The close agreement between glacier fluctuations and tree-growth variations is explicable in terms of similar summer temperature dependent models for tree growth at the tree line and for glacier behaviour^{4,12–14}. The altitude of the equilibrium line, the line dividing the glacier into accumulation and ablation zones, is a climate-sensitive parameter and is particularly influenced by summer temperature^{15,16}. Establishment of the altitudinal displacement of the glacier equilibrium line permits calculation of the temperature difference between the temperature necessary to maintain the present glacier in equilibrium and the temperature that was necessary in ~ AD 1750 (when Storbreen reached its 'Little Ice Age' maximum extent¹⁰). The altitude of the steady-state equilibrium line necessary to maintain Storbreen in equilibrium at present (1949–63) has been established as 1,690 m (ref. 9). Assuming a constant ratio between the accumulation and ablation areas on the glacier in the equilibrium condition, the AD 1750 steady-state equilibrium line was 65–70 m lower than the present (1949–63) steady-state

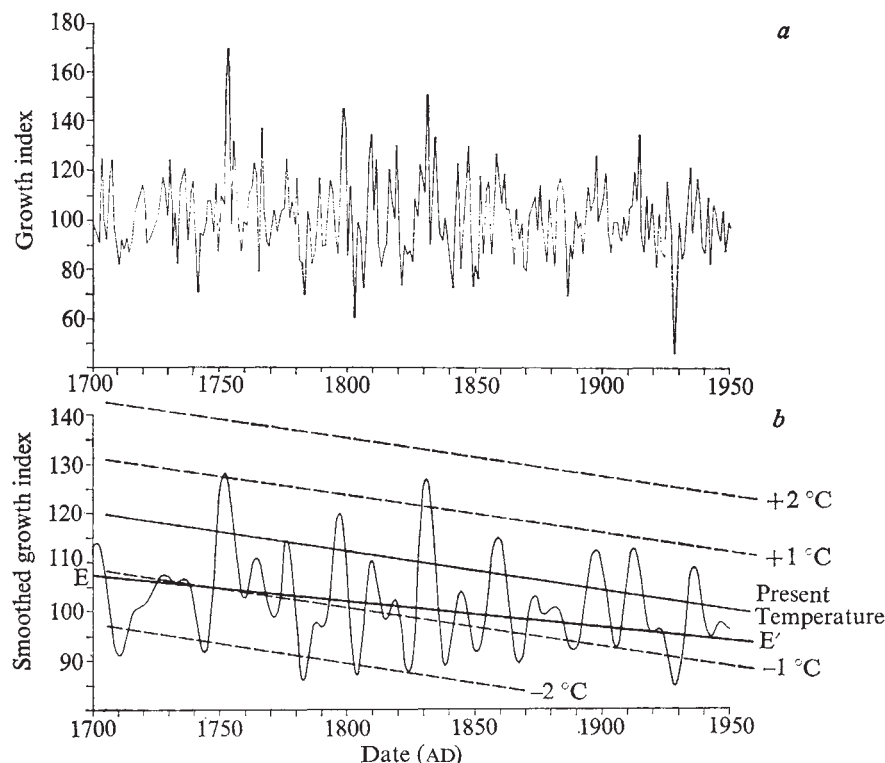
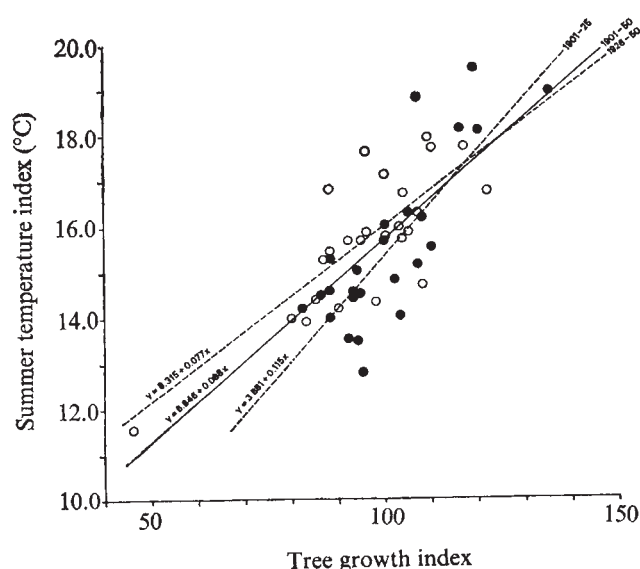


Fig. 2 Graphical representation of the variation in tree growth over the past 250 yr (a) and the smoothed curve after harmonic analysis (b). The smoothed curve is calibrated using an index of summer temperatures, $(1 \times \text{June} + 2 \times \text{July})/3$ and is expressed as differences from the present (1949–63). The line (EE'), which represents the tree-growth value corresponding to Storbreen in the equilibrium condition, was used in the calibration as explained in the text.

line (J. A. Matthews, unpublished). Thus the temperature necessary to maintain the glacier in equilibrium in AD 1750 was $\sim 0.47^\circ\text{C}$ lower than the temperature necessary to maintain equilibrium at present (assuming summer temperature dependence of the equilibrium-line altitude and a summer vertical temperature gradient of 0.7°C per 100 m). On average, between 1949 and 1963, the mean equilibrium line¹⁷ has been ~ 75 m higher than the steady-state line⁹. This means that a fall in temperature of $\sim 0.53^\circ\text{C}$ would be necessary for the present glacier to be in equilibrium and also that in AD 1750, when the glacier was in equilibrium, temperatures were $\sim 1.00^\circ\text{C}$ below present.

The information derived from equilibrium-line displacement permits calibration of the tree-growth variations in terms of summer temperatures. The calibrated curve (Fig. 2b) provides

Fig. 3 The relationship between the summer temperature index, $(1 \times \text{June} + 2 \times \text{July})/3$, and tree growth for the period 1901–50 (—). Relationships for the subperiods, 1901–25 (○) and 1926–50 (●), are also shown (---).



a continuous record of summer temperatures over the past 250 yr. At least 10 major oscillations are indicated, superimposed on a long-term temperature rise of 1.0°C since AD 1750. Cool fluctuations reached 1.0 – 2.0°C below present; warm fluctuations were up to 1.0°C above present temperatures. These temperature fluctuations are regarded as irregular rather than cyclic, although recent work based on the Grampian Mountains, eastern Scotland¹⁸ has suggested the existence of cyclic patterns in growth of Scots pine during the twentieth century.

A brief description of summer temperature changes, reconstructed from tree growth in 25-yr periods since AD 1700, is summarised in Table 1. The first half of the eighteenth century

Table 1 Summer temperatures reconstructed from tree growth during 25-year periods. The temperatures are based on the index $(1 \times \text{June} + 2 \times \text{July})/3$ and are expressed as differences from present (1949–63).

Date (AD)	Index ($^\circ\text{C}$)
1701–1725	–1.63
1726–1750	–1.14
1751–1775	–0.48
1776–1800	–0.87
1801–1825	–1.15
1826–1850	–0.46
1851–1875	–0.55
1876–1900	–0.43
1901–1925	–0.28
1926–1950	–0.34

is shown to have been at least 1.0°C cooler than the present, particularly early in the century when, from 1701–25, summer temperatures were on average 1.6°C below present. In contrast, the period 1751–75 was markedly warmer ($< 0.5^\circ\text{C}$ below present) and corresponds well with the retreat of Storbreen and other southern Norwegian glaciers¹⁹ from their 'Little Ice Age' maxima reached $\sim$ AD 1750. Renewed cooling occurred near the end of the eighteenth century (1776–1800) and continued into the nineteenth century (1801–25), but this cooling phase was not as great as that experienced at the beginning of the eighteenth century. Since 1825 no period has been $> 0.6^\circ\text{C}$ below present temperatures; 1825–1900 was from 0.4°C to 0.6°C cooler than at present and the twentieth century (1901–1950) is indicated as being warmer

than any previous 50- or 25-yr period with summer temperatures that were on average only 0.3 °C below those of 1949–63.

The temperature fluctuations that have been inferred from tree-growth variations are in broad agreement with and supplement existing knowledge from other sources such as instrumental records, stable isotope ratios in ice and wood, treeline fluctuations, glacier fluctuations, the extent of Arctic pack-ice, fluvio-glacial sedimentation rates, the incidence of rockfalls, avalanches and other natural disasters, vine harvest dates and crop failures^{19–25}. The continuous nature of the tree-growth record and the proximity of treelines to glaciers in many geographically separate localities should enable application of similar approaches in other areas and a contribution to be made to knowledge of recent climatic changes in time and space.

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k_{uu} = association constant =

$$\frac{[M_{uu}]}{[M_u]^2} = \frac{[I_{uu}]}{[I_u]^2} \frac{(k_u)^2}{k_{uu}} = \frac{[I_{uu}]}{[I_u]^2} k_i^*$$

k_i^* can vary from one experiment to another due to a change in the source geometry and emitter temperature.

In the temperature range studied, the temperature dependence of k_i^* is negligible. Figure 1 shows temperature dependence of the molecular ion peak intensity I^+ for cytosine, curves 1 and 2 based on electron impact and field ionisation measurements, respectively. The molecular beam was emitted from the orifice of a small Knudsen chamber (Fig. 1, ref. 5), its intensity being proportional to the saturated vapour pressure in the chamber. The heat of sublimation H_s is given⁶ by

$$\frac{d \ln(I^+T)}{d(1/T)} = \frac{-H_s}{R}$$

as 33.8 ± 0.5 kcalorie per mol by both measurements (curves 1 and 2, Fig. 1). On the other hand, the quartz resonator data which do not involve ions⁷ give a value calculated from

$$\frac{d \ln(v \times T^i)}{d(1/T)} = \frac{-H_s}{R}$$

of 34.1 ± 0.3 kcalorie per mol. The agreement between all three values supports the validity of the treatment.

Thus the base-pair binding enthalpy, ΔH , can be found from the temperature dependence of the association constant

$$\frac{d \ln K}{d(1/T)} = \frac{\Delta H}{R} \quad (1)$$

It is, however, necessary to confirm that binding energy determined in this way corresponds to the specific hydrogen bonds responsible for the complementary interaction⁸. The field mass spectra of 1-methyl-U and of dimethyl-U and T are shown

Mass spectrometric studies of binding energies for nitrogen bases of nucleic acids *in vacuo*

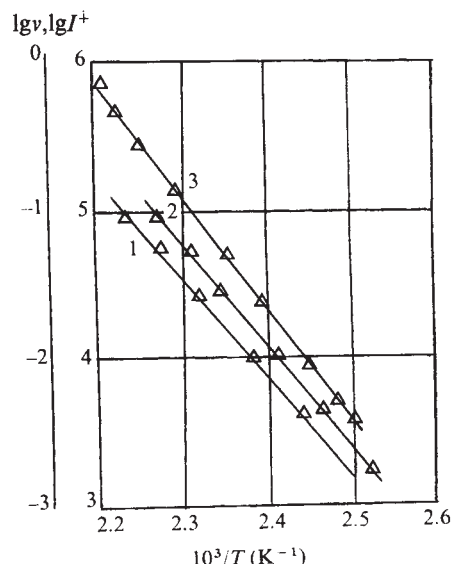
FOR an understanding at the molecular level of the stability and interactions of nucleic acids, including the mechanisms of mutational events, the thermodynamic characteristics of base-pair formation must be determined¹. The most fundamental expression of these characteristics relates to the interaction *in vacuo*. We describe here the measurement of binding enthalpy of a single base pair from various combinations of bases *in vacuo*. We used pyrimidine bases methylated at the 1 position and purine bases methylated at the 9 position. As expected^{2,3}, the results obtained differ appreciably from those of earlier experiments⁴ on solutions, involving indirect methods of observing base pairing.

We have developed a direct method using mass spectrometers with a field ionisation source⁵. The field ionisation mass spectra contained peaks which could be attributed to ionised monomers and dimers only. The peak intensity was proportional to the concentration of the corresponding component near the field emitter. If concentrations are represented by $[M]$ for molecule and $[I]$ for ions then for the case of uracil and its dimer

$$[I_{uu}] = k_{uu}[M_{uu}]; [I_u] = k_u[M_u]$$

where k_{uu} , k_u are ionisation efficiency factors.

Fig. 1 $\ln I^+(1/T)$ and $\ln v(1/T)$ for 1-methylcytosine derived from measurements by: 1, electron impact; 2, field ionisation; 3, quartz resonator method. v , Vapourisation velocity.



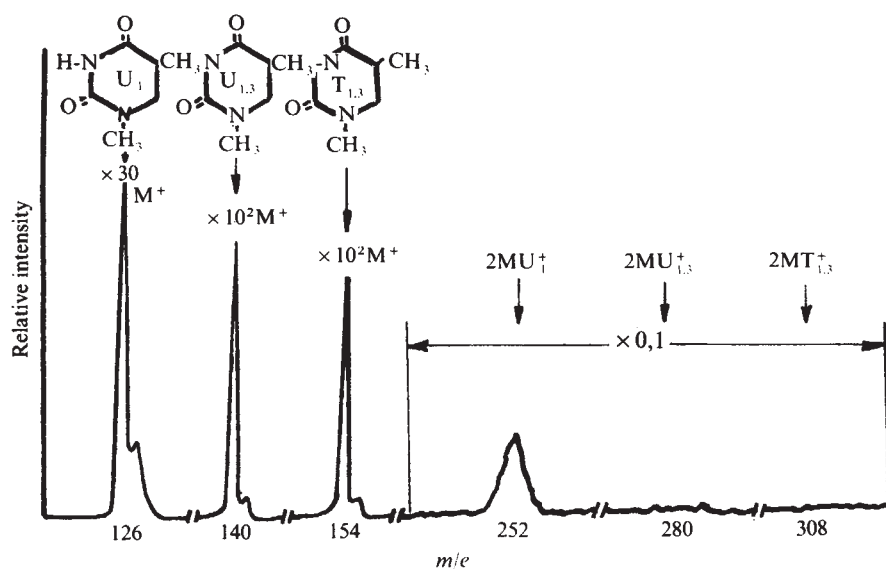


Fig. 2 Field ionisation mass spectra for 1-methyluracil (U_1); 1,3-dimethyluracil ($U_{1,3}$); 1,3-dimethylthymine ($T_{1,3}$). Evaporator temperature was 40 °C for the first and 30 °C for the second samples. Scale factor given for each peak.

in Fig. 2. In the latter two cases hydrogen bonds cannot be formed, and the peaks $2M^+$ are not observed in spite of the almost three times greater concentration of monomers at the emitter. The height of the $(M+1)^+$ peak for doubly methylated bases agrees well with the carbon isotope composition⁹, while for U_1 protonated ions were also observed, indicating that the proton of the 1-nitrogen atom participates in pairing.

The base-pair lifetime of 10^{-6} s (ref. 8) at $t = 25$ °C is much longer than the escape time of the ion from the ionisation zone (10^{-12} s) (ref. 9). Thus base pairing precedes ionisation and occurs between neutral molecules.

Table 1 Enthalpies of base-pair formation

Base pair	$-\Delta H^*$ (kcalorie mol ⁻¹)	$-\Delta H$ (kcalorie mol ⁻¹)		
		(2)	(3)	(4)
AU	14.5	7.21	—	6.2 ± 0.6
UU	9.5	5.42	—	4.3 ± 0.4
AT	13.0	7.0	7.7	—
TT	9.0	5.21	—	—
GC	21.0	16.79	21.4	$10 - 11.5$
CC	12.0	13.72	—	6.3

*The experimental error in ΔH is ± 1 kcalorie mol⁻¹.

The temperature dependence of the association constants in molecular beams of GC and AT is shown in Fig. 3. The curves for AU are similar. The binding energy is found from the slopes of the van't Hoff plots (Fig. 3). The temperature dependence of the relative association constant for the base pairs, observed in the same experiment, is particularly relevant because this is essentially independent of variable factors. This therefore gives a more reliable difference between binding energies for the base pairs in question than the absolute values. The difference so found is -8.5 kcalorie per mol for GC and CC, -5.5 kcalorie per mol for AU and UU and -5.0 kcalorie per mol for AT and TT.

The values of binding energies for various base-pair combinations are summarised in Table 1. The canonical GC pair can be seen to be more stable than the CC pair, while AU and AT are similarly stronger than UU and TT. When our binding energies are compared with theoretical predictions^{2,3}, the agreement is quite good for GC and CC pairs, while the experimental data for pairs containing A, U and T bases are a factor of 1.7 higher than predicted. Our binding energies for pairs *in vacuo* are about double those for bases interacting in chloroform⁴, in which, however, association is weaker than in the inert solvent carbon tetrachloride¹⁰.

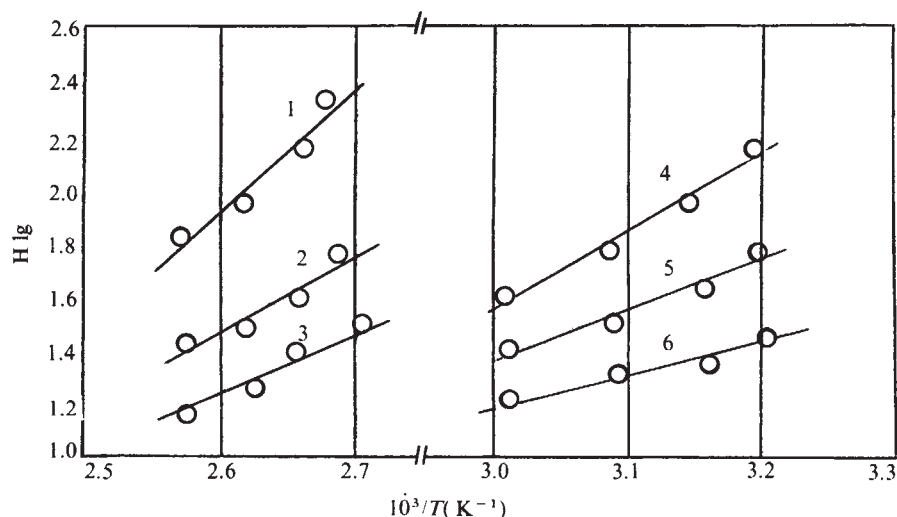


Fig. 3 van't Hoff plots for base-pairing associations: 1, GC; 2, CC; 3, GC-CC; 4, AT; 5, TT; 6, AT/TT.

The relative stabilities of base pairs determined previously⁵ are



This sequence agrees also with the results of dielectric measurement in non-polar solvents⁶, for example at 25 °C in benzene we have



(association constants 3×10^4 , 1.2×10^3 , 150, 28, 15, 8 mol⁻¹, respectively). It is interesting that the extrapolation of the plots in Fig. 3 down to 25 °C yields 10^3 and 40, which agree well with the relative association constants calculated from series (3). Thus our experimental method introduces no appreciable systematic errors.

Comparison of the experimental stability series (2) with theory shows good agreement². The only exception is the CC pair, because according to theory, it should be more stable than the heteroassociates AU and AT. The discrepancies between the stability series (3), and the last column in Table 1, indicating that CC is at least of the same stability as AU and AT, suggest that the binding energy for CC requires further investigation.

Future experiments are expected to yield more accurate enthalpies of pairing of nitrogen bases *in vacuo* as well as data for other base-pair combinations which are important to an understanding of many problems concerning intermolecular and intramolecular interactions in nucleic acids.

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Interaction of heterocellular hereditary persistence of foetal haemoglobin with β thalassaemia and sickle cell anaemia

IN normal adults a small amount of foetal haemoglobin (HbF) is detectable in up to 8% of the red cells (F cells)^{1,2}. In several inherited conditions, which include the Swiss³ and British⁴ types of hereditary persistence of foetal haemoglobin (HPFH), the proportion of F cells is increased in otherwise haematologically normal adults. It has been suggested (S. H. Boyer *et al.*, unpublished) that these conditions should be referred to as heterocellular HPFH to distinguish them from the more well known types of HPFH in which HbF is distributed throughout all the red cells (pancellular HPFH). Here we present evidence that the interaction of a gene for heterocellular HPFH with that for either β thalassaemia (β^{thal}) or sickle cell haemoglobin (HbS) results in the production of significantly greater amounts

of HbF than is usually found in β thalassaemia or sickle cell anaemia alone. The increased output of HbF which results from this interaction reduces the clinical severity of these common disorders. It seems that the genetic determinant for heterocellular HPFH is linked to the $\gamma\delta\beta$ -gene complex.

We have studied three families in which at least one member is a β^{thal} heterozygote with an unusually high level of HbF. The pedigrees of these families, together with the HbF, HbA₂ and F cell values of individual family members, are presented in Fig. 1. Detailed haematological data will be presented elsewhere (W.G.W. *et al.*, unpublished).

The proband of family M, of Italian origin, is a β^{thal} heterozygote with an atypically high HbF level. Her father has typical β^{thal} trait with the slight increase in HbF commonly found in that condition⁵; and both her mother

Table 1 Segregation of heterocellular HPFH and β^{thal} or β^{s} genes among the offspring of double heterozygotes

Family	β -chain marker	Offspring		ref.
		Parental type	Recombinants	
1	β^{thal}	2	0	11
2	β^{s}	2	1	12
3	β^{s}	7	0	13
4	β^{s}	3	0	14
S	β^{s}	8	1	This report
W	β^{thal}	5	1	This report
Total		27	3	

The excess of parental types indicates that the two genes are linked, with three recombinants (family 2, III.2, family S II.3, family W II.1) out of 30 informative offspring.

and brother, who are haematologically normal, also show a slightly increased HbF level and have elevated numbers of F cells of 12.9% and 12.1%, respectively. These latter individuals thus seem to be heterozygous for heterocellular HPFH; it is possible, therefore, that the high level of HbF observed in the proband results from the interaction of the gene for heterocellular HPFH with the β^{thal} gene.

The proband in family S, of Indian origin, has the haematological characteristics of sickle cell- β thalassaemia. Her haemoglobin consists of 68% HbS, 4% HbA, 2.9% HbA₂ and 24% HbF. The HbF is heterogeneously distributed among 73% of the red cells. This value for HbF greatly exceeds that normally found in sickle cell- β thalassaemia. Similar findings were observed in three siblings. The mother has typical β^{thal} trait, whereas the father has the sickle cell trait but also has an increased level of HbF and of F cells. It seems, therefore, that the proband and her three affected siblings have received the HbS and heterocellular HPFH genes from the father and the β^{thal} gene from the mother. The interaction of the three genes have produced a form of sickle cell- β thalassaemia with unusually high levels of HbF. None of these individuals has experienced the clinical symptoms usually associated with this disorder^{5,6}.

The proband of family W, of West Indian Negro origin, has the high HbF- β^{thal} trait, as does one of her children. Five other children have increased numbers of F cells, in four cases associated with increased levels of HbF. Her husband is normal.

In each of these families it seems, therefore, that a gene for heterocellular HPFH (probably identical to that previously described as Swiss HPFH, ref. 3) is segregating. It interacts with the β^{thal} gene to produce levels of HbF in the 6–15% range, and with the β^{thal} and HbS genes to produce HbF levels as high as 25%. How might this interaction result in such high levels of HbF? There is good evidence that, in patients with β thalassaemia or sickle cell anaemia, cells which contain HbF preferentially survive in

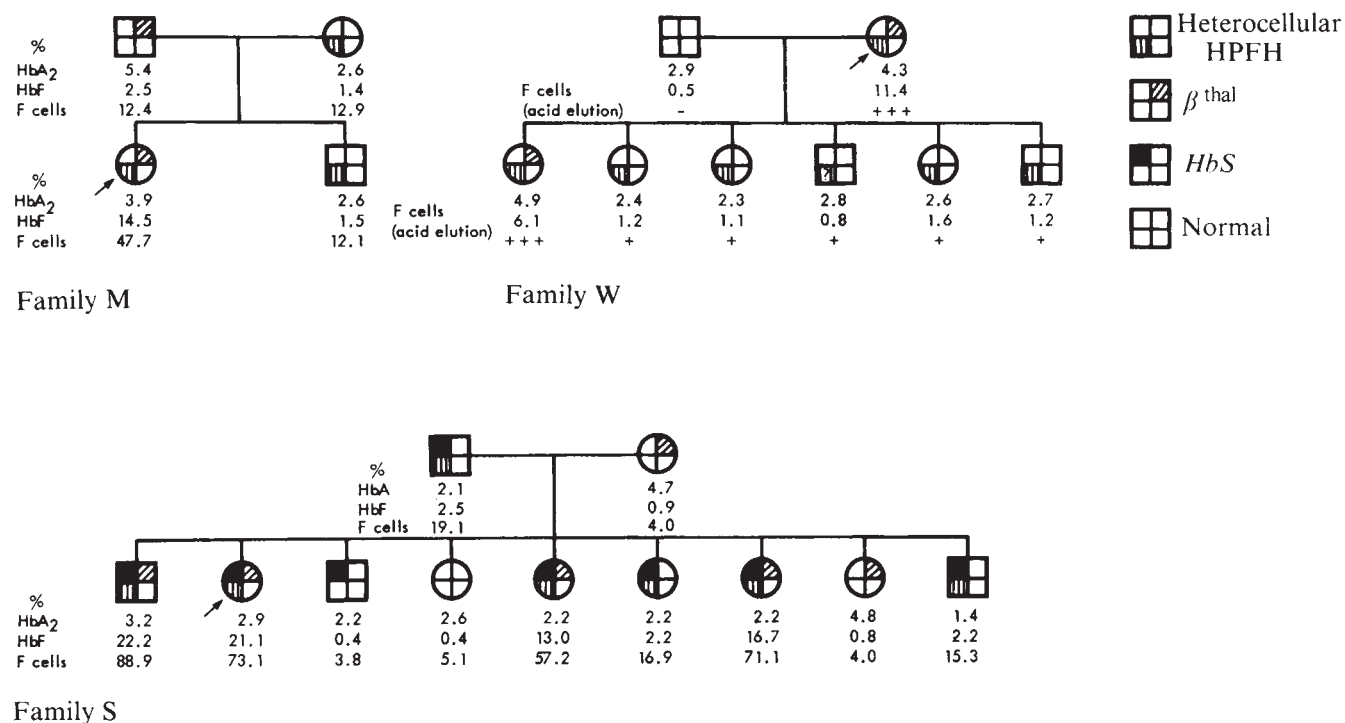


Fig. 1 Pedigrees of three new families in which the gene for heterocellular HPFH is segregating together with that for β thal or HbS. HbA₂ levels were determined by cellulose acetate electrophoresis³, the proportion of HbF was measured by alkali denaturation¹⁷ (upper limit of normal 0.9%), and F cells were measured by immunofluorescence². For family W, semiquantitative estimates of F cell numbers were made in a separate laboratory, using the acid elution technique¹⁸. The genotype of individual II.4 in family W remains in doubt since although his HbF level fell within the normal range, he has more F cells than are found in normal individuals.

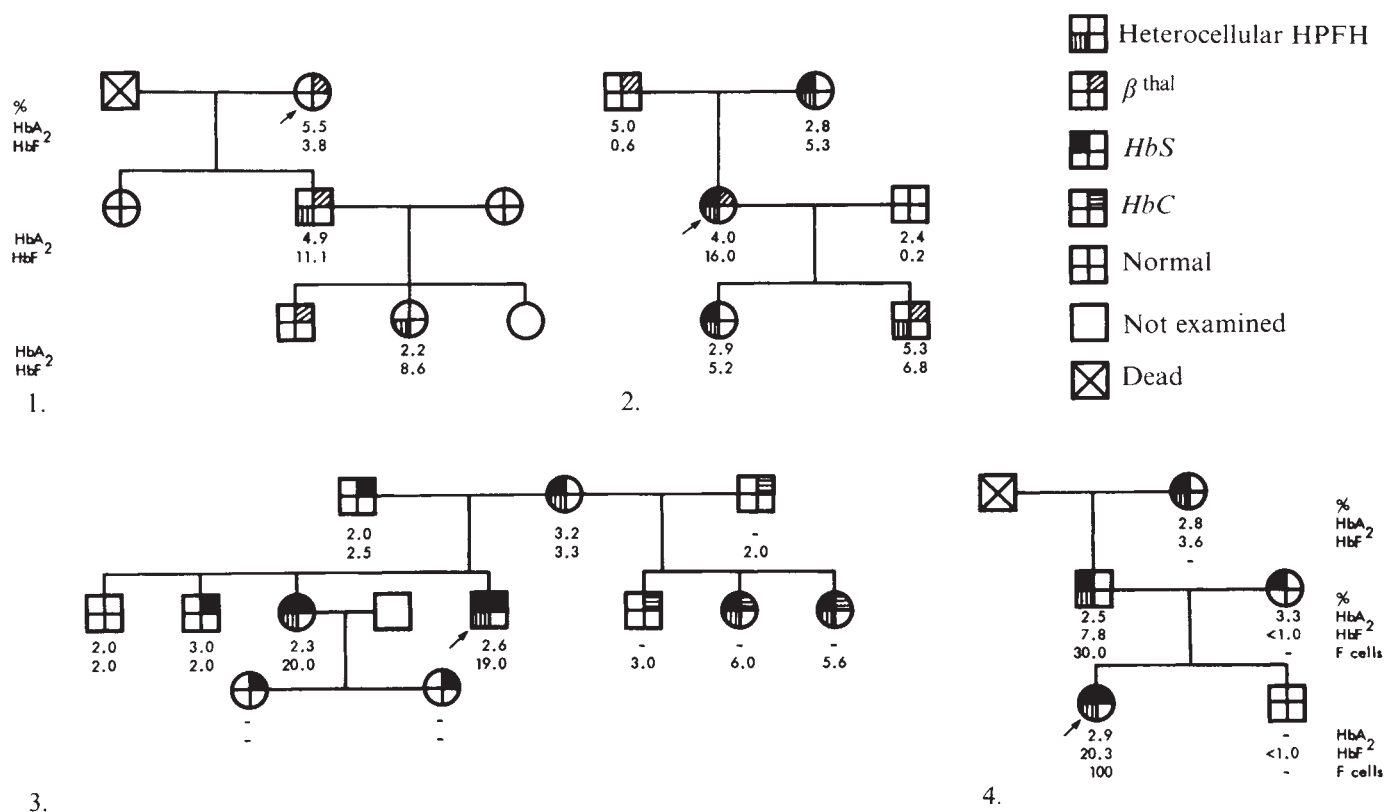


Fig. 2 Pedigrees of four families, previously reported¹¹⁻¹⁴, redrawn to show the interaction of heterocellular HPFH with HbS and/or β thal. The normal range for HbF in some of these reports may be as high as 2.5%. The distribution of HbF in the two HbS homozygotes in family 3 was described as homogeneous¹³. However, the difficulties involved in interpreting the results of the acid elution test¹⁹, and the fact that with 20% HbF most cells should contain HbF, suggest that the high HbF HbS homozygotes in this family can also be interpreted as due to the interaction of heterocellular HPFH with HbS.

the bone marrow or peripheral blood, respectively^{7,8}. It has been suggested that the elevated levels of HbF found in these conditions result from selective survival of the F cell population^{9,10}. If, therefore, in addition to the β^{thal} or HbS genes, there is a gene for heterocellular HPFH increasing the size of the F-cell pool on which the selective processes can act, a high level of HbF should occur. If this is the case, the level of HbF attained will depend on the degree of selection of the F-cell population and therefore on the severity of the condition. The finding of higher levels of HbF in the sickle cell- β thalassaemia heterozygotes than in the β^{thal} heterozygotes is fully in keeping with this prediction.

At least four other families in which it seems that interactions have occurred between the heterocellular HPFH gene and either the β^{thal} or the HbS genes, have been reported previously¹¹⁻¹⁴. Pedigrees of these families, modified according to this interpretation, are shown in Fig. 2. Genetic analysis of these families, together with those which make up the present report, indicate that the gene for heterocellular HPFH is inherited as an autosomal dominant. Furthermore, because the seven families have genes for both heterocellular HPFH and either β^{thal} or HbS segregating, it is possible to test for linkage of the gene for heterocellular HPFH to the β^{thal} or HbS genes. In this analysis we assume that the β^{thal} gene is very closely linked, if not allelic, with the β -structural gene since only one doubtful crossover has been observed in 62 offspring of informative matings⁵. The results, shown in Table 1, indicate that there is a large excess of parental types over recombinants among the offspring and provide good evidence that the gene for heterocellular HPFH is linked to the $\gamma\delta\beta$ -globin gene complex. The frequency of recombination between the gene for heterocellular HPFH and the β -structural gene, three out of 30, compares with no recombinants observed between the δ - and β -structural loci out of 63 offspring of informative matings¹⁵, and at least one, and possibly 3, recombinants between the β^{thal} and δ -structural loci out of 58 informative offspring¹⁵.

The action of the gene for heterocellular HPFH seems to increase the number of F cells rather than the amount of HbF per cell, since there is a linear relationship between the number of F cells and the level of HbF, both in normal adults and in those with heterocellular HPFH (W.G.W. *et al.*, unpublished). Although nothing is known of the mechanism by which this is brought about, it is intriguing that such a controller gene should be linked to the $\gamma\delta\beta$ -structural gene complex. In all these families the gene for heterocellular HPFH has been found *cis* to the β^s gene and *trans* to the β^{thal} gene. We would predict that there should be a marked survival advantage in having the gene for heterocellular HPFH linked *cis* to the β^s locus. The advantage which the β^s gene has in malarious areas would then be maintained without loss of β^s genes in the homozygous state since they would be protected by the high levels of HbF. The mild clinical condition of the HbS homozygotes^{13,14} and HbS- β^{thal} compound heterozygotes¹², who also carry the gene for heterocellular HPFH, confirm these predictions. It will be of great interest to study patients with sickle cell anaemia or homozygous β thalassaemia who have unusually mild courses and high levels of HbF, to determine whether they represent further examples of the interaction of the gene for heterocellular HPFH with these conditions. Such a project is already under way in Saudi Arabia where the Shiite Arab HbS homozygotes have a very mild sickling disorder due to the presence of 15-30% HbF (ref. 16).

The study of heterocellular HPFH is of considerable practical importance because it is clear that if it were possible to increase the F-cell population in patients with sickle cell anaemia or β thalassaemia, the clinical severity of these disorders would be reduced.

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Chromosome 21 does not code for an interferon receptor

It is well established that human cells which are trisomic for chromosome 21 are more sensitive to the antiviral activity of interferon than normal diploid cells¹⁻⁶. If chromosome 21 codes for an interferon receptor site localised at the cell surface, as suggested^{1,3}, one might expect cellular binding of interferon to increase in the order monosomic 21 cells, disomic 21 cells and trisomic 21 cells. This did not prove to be the case. All cell types bound interferon equally well. In view of these and previously⁵ reported data, it is postulated that chromosome 21, if it codes for a protein involved in the interferon response of the cells, does not specify the receptor molecule *per se* but another molecule that is involved in the processing of the antiviral message from the cell surface to the interior of the cell.

Human interferon, whether induced by viruses, double-stranded RNA or phytohaemagglutinin in either leukocytes, fibroblasts or lymphoblasts, seems to elicit a higher antiviral response in human skin fibroblasts which are trisomic for chromosome 21 (T-21) than in cells which are disomic for chromosome 21 (D-21); D-21 cells are in turn more sensitive to the antiviral action of interferon than cells which are monosomic for chromosome 21 (M-21)¹⁻⁶. To account for the increased interferon sensitivity of T-21 cells, different hypotheses have been proposed. According to Tan^{1,2}, chromosome 21 governs the synthesis of a secondary antiviral protein, whereas Chany⁴ postulated that chromosome 21 would carry the structural genes for an interferon receptor. From studies with interferon covalently bound to an insoluble matrix it was inferred that the interferon receptor site may be located at the outer cell membrane⁷⁻¹⁰. That such a receptor site could be specified by chromosome 21 was suggested by Revel *et al.*⁵. They found that an antiserum against a chromosome 21-directed cell-surface component reduced markedly the antiviral action of interferon in normal human diploid cells.

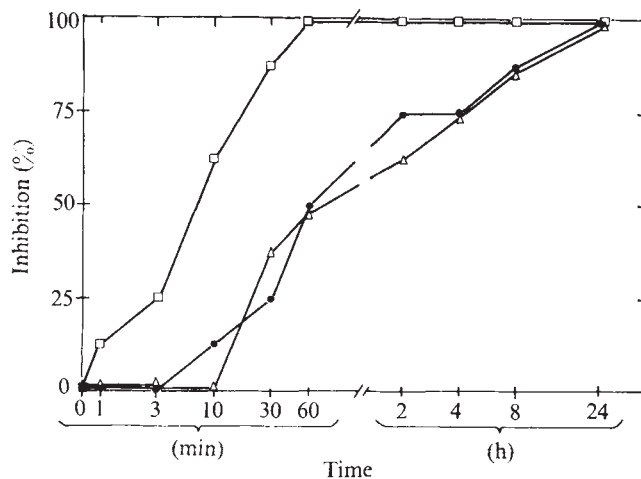


Fig. 1 Inhibition of CPE in T-21, D-21 and M-21 fibroblast cultures exposed to human leukocyte interferon (100 IU ml^{-1}) for varying times (as indicated in the abscissa) and challenged at 24 h with VSV. Human fibroblast cultures, which were either trisomic for chromosome 21 (T-21) (designated SCH), disomic for chromosome 21 (D-21; designated VGS) or monosomic for chromosome 21 (M-21; designated GM-230), were grown to confluency in Eagle's MEM+10% FCS in micro Linbro plates. When confluent, the cells were exposed to 100 IU ml^{-1} human leukocyte interferon (in Eagle's MEM+3% FCS) for either 1, 3, 10, 30 or 60 min, or 2, 4, 8 or 24 h. After incubation with interferon the cells were drained, washed and further incubated with Eagle's MEM+3% FCS. At 24 h all cell cultures were challenged with 100 CCID₅₀ (cell culture infecting dose 50) of VSV and viral CPE readings were made as soon as CPE reached 100% in the virus controls: this was, for a large number of experiments, at 1 d in GM-230 cells, at 2 d in VGS cells and at 2–3 d in SCH cells. □, T-21 (SCH); ●, D-21 (VGS); △, M-21 (GM-230).

If chromosome 21 indeed codes for the interferon receptor, one may expect T-21 cells to be more sensitive to all activities of interferon, both antiviral and non-antiviral. Our data³ indicate, however, that, unlike the antiviral activity, the non-antiviral effects of interferon "priming" and "toxicity enhancement" are not better expressed in T-21 than in normal diploid cells. These findings are difficult to reconcile with an interferon receptor coded for by chromosome 21, unless one assumes the existence of two (or more) interferon receptor sites, one of which is responsible for the antiviral state and is controlled by chromosome 21, and a second one which is not determined by chromosome 21 and mediates (some of) the non-antiviral effects of interferon.

If the assumption that chromosome 21 codes for an interferon receptor is correct, one might expect cellular binding of human interferon to increase in the order M-21, D-21 and T-21, the more since binding of interferon to the cells seems to be related to the antiviral and anticellular action of interferon in these cells^{11–13}. Therefore, cell binding of interferon was examined with M-21, D-21 and T-21 cells, and, to minimise nonspecific binding, the cells were exposed to interferon for a limited time period (30 min). In concomitant experiments it was ascertained that such short incubation enabled part of the antiviral potency of interferon to be expressed.

The sources of human leukocyte and fibroblast interferon were the same as those referred to previously³. All human fibroblast cultures were derived from skin biopsies. The monosomic 21 lines (designated GM-137 and GM-230) were obtained from the Mammalian Genetic Mutant Cell Repository (Camden, New Jersey). The latter cell lines were also employed in Tan's studies². All cell cultures were matched as nearly as possible with regard to the number of cell generations in culture.

To measure resistance to virus infections, cells are generally incubated with interferon for a 18–24-h period before virus challenge. In such conditions T-21 fibroblasts are more

sensitive to the antiviral action of interferon than normal diploid fibroblasts, which are in turn more sensitive than M-21 fibroblasts^{2,3}. When the time of exposure of interferon to the cells was shortened, interferon activity gradually diminished (Fig. 1). Irrespective of the time the cells were left in contact with interferon before virus challenge, T-21 cells proved invariably more sensitive to the antiviral activity of interferon than D-21 or M-21 cells—for example, a 30-min exposure of T-21 cells to 100 U ml^{-1} of interferon afforded nearly complete protection against the cytopathic effect of vesicular stomatitis virus (VSV). To attain a similar protection in D-21 and M-21 cells, an 8-h interferon treatment was required (Fig. 1). Contrary to what may have been expected from a gene dosage effect², M-21 and D-21 cells were equally sensitive to the antiviral action of interferon (Fig. 1). It should be pointed out that viral cytopathic effect (CPE) developed faster in M-21 than in D-21 cells (whether the differences in rate of CPE development reflect differences in the kinetics of virus multiplication is now being studied). Therefore, CPE readings were made at an earlier time for M-21 than for D-21 and T-21 cells. This may explain the differences between our results and those of Tan², who estimated viral RNA synthesis for all cell types at the same time (8 h after infection).

In T-21 cells, the same interferon titre was attained whether the cells were inoculated with VSV 24 h or immediately after a 30-min interferon treatment. Such a short interferon pulse, whether carried out at 37 (Table 1) or 4 °C (data not shown) resulted in the expression of 3–10% of the total interferon activity in T-21 cells and 0.3–1% of the total interferon activity in M-21 and D-21 cells (Table 1). Again, in conditions in which virus challenge was applied immediately after a 30-min interferon treatment, no significant differences were observed in the behaviour of D-21 and M-21 cells. Thus, no gene dosage effect² could be observed in our assay conditions.

Human fibroblast cultures derived from old donors behave very much like T-21 cells in their sensitivity to the antiviral action of interferon¹⁴. The differential sensitivity

Table 1 Interferon titre expressed in various T-21, D-21 and M-21 fibroblast cultures exposed to human leukocyte interferon or human fibroblast interferon (10^4 IU ml^{-1}) for 30 min at 37 °C, and then challenged immediately with VSV

Cell type	Designation	Interferon titre ($\log_{10} \text{ IU ml}^{-1}$)	
		Leukocyte interferon	Fibroblast interferon
T-21	SCH	2.8 (2.5–3.0)	2.5
	BR	3.0	2.5
Old D-21	SW	2.5	2.3
	G	2.5	2.3
D-21	VGS	1.8 (1.5–2.0)	1.6 (1.5–1.8)
	VG ₃ S	2.0	1.5
M-21	GM-230	1.7 (1.5–2.0)	1.5
	GM-137	2.0	1.5

Human fibroblast cultures, which were either trisomic for chromosome 21 (T-21; designated SCH and BR), disomic for chromosome 21 (D-21) and derived from old donors (designated SW and G, both 80 yr old), disomic for chromosome 21 (D-21) and derived from young donors (designated VGS and VG₃S), or monosomic (M-21) for chromosome 21 (designated GM-230 and GM-137), and which had been grown to confluency in micro Linbro plates, were exposed to serial dilutions (in Eagle's MEM+3% FCS) of human leukocyte or fibroblast interferon stock preparations, both containing 10^4 IU ml^{-1} . The interferon incubation period was limited to 30 min at 37 °C. Immediately afterwards, the cells were washed and challenged with 100 cell culture infecting dose 50 (CCID₅₀) of VSV. The interferon titre was determined as the reciprocal of sample dilution inhibiting viral CPE by 50%. CPE readings were taken as soon as viral CPE in the control cell cultures reached 100%. The data show means for 3–10 experiments. The range of the individual values is indicated in parentheses.

Table 2 Binding of interferon to T-21, D-21 and M-21 fibroblast cultures exposed to human leukocyte interferon or human fibroblast interferon for 30 min at 37 °C

Interferon applied to the cells (log ₁₀ IU ml ⁻¹ per Petri dish)	Interferon recovered from culture medium (log ₁₀ IU ml ⁻¹ per Petri dish)			Interferon recovered from cells (log ₁₀ IU ml ⁻¹ per 5 Petri dishes)		
	T-21	D-21	M-21	T-21	D-21	M-21
Human leukocyte interferon						
3.0	2.8	2.8	2.8	1.2	1.2	1.0
4.0	3.6	3.6	3.3	1.4	1.4	1.3
5.0	5.2	5.2	5.2	2.7	2.5	2.5
Human fibroblast interferon						
3.0	2.5	2.2	2.5	1.0	1.0	0.7
4.0	3.4	3.3	3.3	1.2	1.1	1.0
5.0	4.5	4.5	4.5	1.5	1.7	1.3

Human fibroblast cultures, which were either trisomic for chromosome 21 (T-21; designated SCH), disomic for chromosome 21 (D-21; designated VGS) or monosomic for chromosome 21 (M-21; designated GM-230) and which had been grown to confluency in 60-mm Falcon Petri dishes, were exposed to human leukocyte or fibroblast interferon (10^3 , 10^4 or 10^5 IU ml⁻¹ in Eagle's MEM+3% FCS). The interferon incubation period was limited to 30 min at 37 °C. Immediately afterwards, the culture medium was collected for assay of residual interferon activity and the cell monolayer was washed three times with Dulbecco's PBS at 4 °C. The cells were then scraped off with a rubber policeman into 0.5 ml PBS per Petri dish, pelleted and resuspended with 1 ml PBS per 5 Petri dishes. Cell homogenates were prepared by ultrasonication for 20 s in a 100 W ultrasonic disintegrator MSE (operating at maximum output and at a nominal frequency of 20 KHz). These cell homogenates were assayed for interferon content. All interferon titrations were carried out using a VSV CPE inhibition assay in D-21 (VGS) cells. Approximate number of cells per Petri dish: 10^6 (for T-21, D-21 and M-21 cells). Average protein content per Petri dish (determined by the Lowry method): 852 µg (range: 550–1,080 µg) for T-21; 985 µg (range: 640–1,240 µg) for D-21; and 625 µg (range: 520–720 µg) for M-21 cells.

of 'old' and young' diploid fibroblasts to the antiviral effect of interferon was also observed in the present experiments in which the cells were pulse-treated with interferon before virus challenge (Table 1).

The rapid action of human interferon in T-21 and old D-21 cells (Table 1) is reminiscent of the rapidly developing resistance to virus infection induced by interferon in what has been considered to be physiological conditions¹⁵.

Is the enhanced antiviral action of interferon in T-21 cells, as noted after a brief exposure of the cells to interferon, mediated by or, at least related to an increased binding of interferon to the cells? To assess this question, cell binding of interferon was examined in conditions which allowed a clear differentiation between the antiviral activities of interferon in T-21 and D-21 cells. Therefore, uptake of interferon by the cells was measured after a 30-min contact period at 37 °C. The amount of cell-bound interferon seems to reach a plateau after a 30-min incubation period¹². Cell-associated interferon as well as residual supernatant interferon were quantified for T-21, D-21 and M-21 cells which had been exposed to various doses (10^3 , 10^4 or 10^5 U ml⁻¹) of either human leukocyte or fibroblast interferon. As shown in Table 2, very small amounts of interferon (0.01–0.1%) were taken up by the cells. The amounts of interferon taken up by T-21 cells did not differ markedly from the amount of interferon which became associated with either M-21 or D-21 cells (Table 2).

Thus, the increased sensitivity of T-12 cells to the antiviral action of a 30-min interferon pulse is not reflected by an increased binding of interferon to these cells. Such increased binding would have indicated an increased number of available interferon receptors and/or an increase in the affinity of such receptors for interferon. Provided that our data do not support the existence of an interferon receptor site coded for by chromosome 21, how could they be reconciled with the implication of a chromosome 21-specified cell surface component in the antiviral action of interferon? Hypothetically, the cellular component coded for by chromosome 21 is not the interferon receptor *per se*, but part of a molecular complex that is involved in the processing of the antiviral message from the cell surface to the interior of the cell. The regulator function of chromosome 21 would be required for the antiviral action of interferon, but not for its other effects such as "priming" and "toxicity enhancement"³. Since D-21 cells derived from old donors (ref. 14 and Table 1) and some other D-21 cells (for example, cells derived from cystinosis patients⁴) behave like T-21 cells in their sensitivity to the antiviral action of

interferon, whereas triploid cells (which are trisomic for all chromosomes) act like D-21 cells⁴, the question may even be raised as to whether chromosome 21 directly codes for any protein involved in the interferon response of the cells.

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Episome-like behaviour of donor DNA in transformed strains of *Neurospora crassa*

TRANSFORMATION is a well established process for the transfer of genetic information in bacteria^{1,2}. Although such a system in higher organisms remains to be fully elucidated, certain features of gene transfer in eukaryotes have similarities with bacterial transformation³. The occurrence of inositol-independent (*inl*⁺) transformants has been reported in *Neurospora* when an inositol requiring (*inl*⁻) mutant strain (89601) is treated with a wild-type (RL3-8A) DNA preparation^{4,5}. Although these transformants are stable for inositol independence (*inl*⁺) during the somatic cell cycle they differ in the ability to transmit this character to their sexual progeny. Essentially there are two groups of transformants, one capable of Mendelian transmission

and another showing only a rare non-Mendelian transmission of the transformed character (*inl*⁺)⁵ (Table 1). Such differences in modes of genetic transmission can be explained if the donor DNA exists as an episome in the transformed strains. Thus the physical status of the episomes determines the Mendelian and non-Mendelian transmission of the genetic character controlled by them. When integrated they are transmitted in a Mendelian way like any other chromosomal gene; whereas in the non-integrated (autonomous) form their nucleolytic degradation (or slow replication followed by segregation) during meiosis can lead to non-Mendelian transmission. This hypothesis is supported by demonstration of a continued aberrant transmission of the inositol independence to the sexual progeny in the subsequent generations⁵. Here we describe experiments which establish further the validity of the above hypothesis.

We used DNA-intercalating drugs to eliminate the non-integrated episomes from the transformed strains of *Neurospora*. Both ethidium bromide and acridine have been used

Table 1 Summary of the *Neurospora* strains used

Strains	Description
Wild type (RL3-8A)	No growth requirement for inositol (<i>inl</i> ⁺)
Mutants (89601 a/A)	Require inositol for growth (<i>inl</i> ⁻)
Transformants*	
Group I	
26-2	These strains show Mendelian transmission of the transformed character (<i>inl</i> ⁺) to their meiotic progeny
165	
169	
Group II	
26-6	These strains are rarely able to transmit their <i>inl</i> ⁺ character to their sexual progeny and thus show non-Mendelian transmission
166	
Others	
26-6-1	Require inositol for growth (<i>inl</i> ⁻) and were obtained after treatment of the transformed strain 26-6 with ethidium bromide (10 µg ml ⁻¹)
26-6-2	
26-6-3	

*These strains were obtained as inositol-independent (*inl*⁺) colonies after treatment of the mutant strain (89601) with the wild-type (RL3-8A) DNA preparation^{4,5}. These strains also carry another genetic marker (*os*⁻) and cannot grow on 1 M NaCl. Their origin has been described elsewhere⁵.

successfully to induce loss of autonomous episomes from bacteria⁶⁻⁸ and their mode of action is well known⁹⁻¹¹. The drug-induced loss of episomes in *Neurospora* would lead to the production of cells requiring inositol for growth; such *inl*⁻ colonies can be detected easily in *Neurospora* by conidial analysis. When treated with these drugs, however, only transformants showing non-Mendelian transmission of the *inl*⁺ character would be expected to yield *inl*⁻ colonies, whereas the wild-type strain and the transformants showing Mendelian transmission would remain unaffected. Following this rationale, the different *inl*⁺ strains of *Neurospora* were treated with the drugs. The results of the experiments presented here support the hypothesis of episome-like behaviour by the donor DNA in the *inl*⁺ transformants.

The strains of *Neurospora crassa* used are described in Table 1. Cultures of *inl*⁺ strains were treated with different concentrations (0–100 µg ml⁻¹) of ethidium bromide or acridine. The latter was a mixture of 2,8-diamino-10-methyl acridinium chloride and 2,8-diamino acridine (Sigma). The drug was added to a culture at its inception and each culture was then incubated for 4–5 d in the dark at 25 °C. The mycelial mat thus obtained was washed thoroughly and transferred to complete agar medium and allowed to conidiate by incubation for a further 4–5 d. Conidia from each culture were collected separately and plated on complete agar medium containing L-sorbose¹². The growing colonies were then isolated and tested for growth requirement by spreading on minimal medium and on medium supplemented with inositol (80 µg ml⁻¹). The colonies able to grow on minimal medium were scored as *inl*⁺ (their

growth was not affected by the presence of inositol in the medium). Those which required inositol for growth and could not grow on the minimal medium were scored as *inl*⁻. The frequency of production of *inl*⁻ colonies was considered to be a direct measure of the drug-induced loss of the *inl*⁺ determinants. The data presented in Tables 2 and 3 show that the drugs produced *inl*⁻ colonies in significant numbers only from the transformants which showed a non-Mendelian transmission of the transformed character (that is group II). Ethidium bromide was effective in this respect on both strains 26-6 and 166 of this group (Tables 2 and 3). The data in Table 2 also suggest that the loss of inositol independence depended on the concentration of ethidium bromide. The frequency of production of *inl*⁻ colonies increased linearly with increasing drug concentration (Table 2): 50 µg ml⁻¹ was found most effective, and approximately 8% of treated colonies were *inl*⁻.

The effect of acridine was similar to that of ethidium bromide. The data in Table 3 show the production of *inl*⁻ colonies in a significant number by the group II transformants (strains 26-6 and 166) treated with acridine. The effect of acridine also depended on the concentration of drug used (data not shown). The drug-induced loss of the *inl*⁺ character among the group II transformants suggests that in these transformants the donor DNA exists as an autonomous episome (which can be eliminated by treatment with DNA-intercalating drugs).

For comparison, the group I transformants (that is, strains 26-2, 165 and 169) were treated with ethidium bromide and acridine. The data in Table 3 show that their inositol independence was unaffected by drug treatment; no colony which required inositol for growth was produced by such treatment. These data suggest that in the group I transformants the donor DNA (carrying the *inl*⁺ genetic information) has been integrated into the recipient chromosome. As another control, the wild-type strain (RL3-8A) was treated with both of the drugs and, among a large number of the colonies examined, all were found to grow on minimal medium (Table 3). Thus the wild-type strain could retain its inositol independence (*inl*⁺) after drug treatment as expected since the *inl*⁺ genetic information is chromosomal in this strain. The fact that the inositol independence of the wild-type strain was not affected by the drug rules out the possibility of a mutagenic effect of the drug on other strains. Crosses were performed to establish the allelism of the newly isolated *inl*⁻ strain (26-6-1, 26-6-2, and 26-6-3, Table 1) with the parental *inl*⁻ strains (89601). The new *inl*⁻ strain in crosses with 89601 (*inl*⁻) produced only *inl*⁻ progeny; not one wild-type progeny was detected even when more than a million spores were tested from each of these crosses. Also the new *inl*⁻ strains in crosses with the wild-type strain were found to produce asci with four *inl*⁻ and four *inl*⁺ spores per ascus. These genetic data

Table 2 Ethidium bromide-induced loss of the inositol independence (*inl*⁺) in a transformed strain (26-6) of *Neurospora crassa*

Drug concentration (µg ml ⁻¹)	No. and phenotype of colonies obtained after the drug treatment		Frequency of the colonies which lost <i>inl</i> ⁺ character (%)	
	<i>inl</i> ⁺	<i>inl</i> ⁻	Total	
0	886	0	886	0
1	500	0	500	0
5	494	6	500	1
10	581	17	598	2.9
20	473	27	500	5.4
50	459	41	500	8
100	463	37	500	7.5

The transformed strain 26-6 was treated with the different concentrations of ethidium bromide and the colonies obtained by the conidial analysis were examined for their growth requirement with respect to inositol (see text).

Table 3 Effect of drug treatment on the loss of inositol independence (*inl*⁺) among different strains of *Neurospora crassa*

Strains	No. and phenotype of colonies examined after treatment with Ethidium bromide* (10 µg ml ⁻¹)			Acridine* (15 µg ml ⁻¹)		
	<i>inl</i> ⁺	<i>inl</i> ⁻	Total	<i>inl</i> ⁺	<i>inl</i> ⁻	Total
Wild-type strain (<i>inl</i> ⁺) (RL3-8A)	2,560	0	2,560	2,700	0	2,700
Transformed strains (<i>inl</i> ⁺)						
(I) showing Mendelian transmission of the <i>inl</i> ⁺ character						
26-2	943	0	943	500	0	500
165	842	0	842	900	0	900
169	921	0	921	800	0	800
(II) Showing non-Mendelian transmission of the <i>inl</i> ⁺ character						
26-6	662	18	680	1,649	41	1,680
166	689	11	700	587	13	600

*The different inositol-independent (*inl*⁺) strains of *Neurospora* were treated with the drugs separately and then examined by conidial analysis as described in the text.

clearly establish that the *inl*⁻ phenotype of the drug-treated culture was determined by a Mendelian gene and that latter was allelic to the parental *inl*⁻ strain (89601).

An episome can exist inside the recipient cell in two distinct forms, the autonomous state and the integrated state¹³, and the data presented here are compatible with the hypothesis that donor DNA exists as an episome in the transformed strains of *Neurospora*. These data suggest the difference in the physical status of the donor DNA (carrying the *inl*⁺ genetic information) in the two groups of the *Neurospora* transformants. In the group I transformants the episome is chromosomal whereas in group II it is autonomous. Although acridines are known mutagens¹⁴, the effect of ethidium bromide and acridine reported here cannot be explained on the basis of a mutation at the *inl*⁺ locus in the transformants because these drugs did not produce *inl*⁻ colonies in the wild-type strain. Because the transformed strains were stable during their somatic cell cycle it seems that the donor DNA may exist as covalently closed circles to avoid nucleolytic digestion. These, however, could still be liable to digestion by restriction endonucleases during meiosis; the existence of such endonucleases in higher organisms has been invoked to provide the molecular basis of non-Mendelian inheritance in higher organisms^{15,16}. The frequency of elimination of episomes carrying *inl*⁺ genetic information in *Neurospora* was rather low (8%) compared with the high frequency of elimination (up to 100%) of F factors reported earlier^{7,17}. These values in *Neurospora*, however, are comparable with that of the elimination of the drug resistance factors in bacteria¹⁸.

The data presented here imply certain similarities in the behaviour of episomes in prokaryotes and eukaryotes as suggested previously¹⁹. Some of the characteristics of genetic transformation in *Neurospora* described here have some parallels in other eukaryotes²⁰.

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Genetic polymorphism of C4 in man and localisation of a structural C4 locus to the HLA gene complex of chromosome 6

NATIVE C4, the fourth component of human complement (C4)¹ has a molecular weight of 204,000 and is composed of three distinct polypeptide chains linked by disulphide bonds and non-covalent forces². C4 migrates as a β 1 globulin when subjected to electrophoresis in Agarose gel. Genetically determined structural polymorphism has been detected in the complement components C3 (refs 3 and 4), C6 (ref. 5) and properdin factor B (ref. 6). As well as their use in forensic science and population genetics, such polymorphisms are valuable tools for work on genetic linkage and chromosome mapping, as shown by the assignment of the factor B locus to chromosome 6, closely linked to HLA-B^{7,8}. We report here similar findings with human C4.

EDTA-plasma, heparin-plasma and serum were used initially. Samples were either studied within 2 h of collection or stored at -75 °C until examined. Serum proteins were separated by high voltage electrophoresis in Agarose gel⁹ for 1-1.5 h. C4 bands were then made visible by immunofixation with specific antibody¹⁰, and staining with Coomassie brilliant blue. Specific anti-C4 was produced by immunising rabbits with C4 purified by ion exchange chromatography, ammonium sulphate precipitation and preparative electrophoresis on polyacrylamide gel. This monospecific anti-C4 showed reactions of complete identity with anti-C4 obtained from Dr C. Alper, Boston, and also with commercial anti-C4 (Behring Institut and Atlantic Antibodies).

Ninety-three unrelated Norwegian adults have been studied. In 91 of them, one of three patterns was found: one slow (S) band only, one fast (F) band only, or the two together (FS) (Fig. 1). In two individuals a variant pattern was observed in which the ordinary F band occurred together with a band (M) localised between the position of

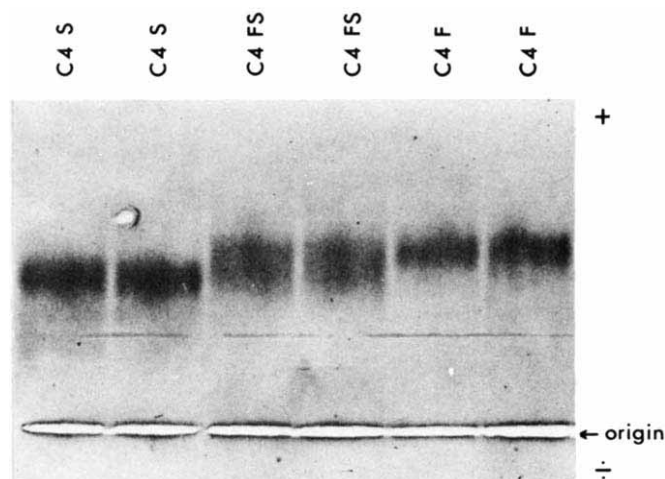


Fig. 1 The common C4 phenotypes revealed by immunofixation electrophoresis.

the S and F bands. These patterns could be reproduced in the same samples examined on different occasions, and in samples from the same individuals bled at different times. The patterns were identical in serum, EDTA-plasma and heparin-plasma when these were handled as described above.

The family data consist of 17 matings with 112 children. The families have been typed for approximately 20 other genetic marker systems, thus assuring a regular pattern of inheritance. Heparin-plasma samples stored at -75°C were typed and the results confirmed the postulate that the bands S and F observed in the material of unrelated adults represent the expression of two alleles $C4^S$ and $C4^F$ at one locus—that is a structural locus for C4 (Table 1).

Among 93 unrelated Norwegians the following gene frequencies were observed: $\sim 0.52 C4^S$, $\sim 0.47 C4^F$ and $\sim 0.01 C4^M$. Phenotype frequencies were in agreement with the expected Hardy-Weinberg distribution (23 C4S, 2 C4FM, 51 C4FS and 17 C4F). Preliminary findings with an extended population sample suggest the existence of other variant alleles.

Table 1 C4 types in 17 matings with 112 children

Mating type	No. of matings	No. of children	C4 types of children				
			S	FS	F	FM	MS
S $\times$ FS	2	22	8	14			
S $\times$ F	1	4		4			
FS $\times$ FS	2	6		4	2		
FS $\times$ F	9	60		25	35		
F $\times$ F	2	8			8		
FS $\times$ FM	1	12		3	2	3	4
	17	112	8	50	47	3	4

The heterogeneity of human C4 has been reported before^{11,12}, and has been postulated to have, at least in part, a genetic background. Gene frequencies, however, have not been reported, and no extensive family studies have been done. It is therefore difficult to assess whether this heterogeneity is related to the polymorphism described here.

The detection of a structural polymorphism gave us the opportunity to try and link the C4 locus to other loci of the human genome. In 85 HLA-B/C4 informative meioses there were no recombinations. This definitely proves that the two loci are closely linked, the recombination fraction being 0.0–4.3 (95% confidence limits). Two informative families with known crossovers between the A and B loci of the HLA region, moreover, indicate that the C4 locus is situated close to the B locus, as shown for the properdin factor B locus⁸.

A patient has been reported with no C4 in the serum, and

a study of the family suggested that the locus determining the level of C4 was closely linked to the HLA region¹³. Our findings suggest that the familial defect in C4 levels is due to a "silent" allele at the structural C4 locus.

The structural locus of properdin factor B, and loci determining the blood levels of C2 (ref. 14) and C8 (ref. 15) have been assigned previously to the HLA gene complex of chromosome 6. It is therefore not surprising that C4 is also encoded by a locus in this complex. It has been shown that the murine C4 locus is situated in the H-2 gene complex^{16,17}. The C3 locus in man is, however, not linked to HLA¹⁸.

In this context it is tempting to speculate that loci for different components of complement have appeared, during evolution, from one locus as results of local chromosomal events, for instance gene duplications and point mutations. Indeed it has been postulated that complement components have evolved from a single ancestral protein⁷.

An interesting pattern emerges with respect to the role of this short segment of chromosome 6 in the immunobiology of vertebrates. It seems significant that many important factors in immune recognition, response and reaction are encoded by closely linked genes. The closeness at the chromosomal level may reflect functional integration of products of immune response genes, cell membrane allo-antigens and the complement system.

Note added in proof: Recently extended population material indicates that the frequency of the S gene may be appreciably lower than in the small sample presented here (~ 0.40).

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Persistence of anti-immunoglobulin on the lymphocyte surface

It is well established that addition of anti-immunoglobulin (anti-Ig) to lymphocytes expressing Ig receptors produces "capping" followed by endocytosis or "sloughing" of the membrane Ig, the expression of which is greatly diminished until resynthesis occurs¹. Another property of anti-Ig in some species, notably the rabbit, is blastogenic activation of lymphocytes². It is known that for the activation of lymphocytes by plant mitogens, the mitogenic substance must persist on the cell membrane for several hours before irreversible activation of the cell occurs³. Similarly, the activation of rabbit lymphocytes by antiserum to allotypic Ig determinants is largely inhibited if the Ig expressing the target allotype is added within a few hours⁴. Thus there is an apparent conflict between these two phenomena, one of which centres around the rapid removal of membrane Ig, the other requiring the persistence of anti-Ig for longer periods. Accordingly, experiments were designed to test the degree of retention of anti-Ig and the re-expression of membrane Ig on the surface of lymphocytes treated with anti-Ig sera and retained in tissue culture for several days.

To study the fate of anti-allotype antibody on rabbit lymphocyte membranes *in vitro*, peripheral blood lymphocytes, homozygous for *b*-locus allotype determinants, were used. These lymphocytes were obtained from defibrinated blood by a gelatin sedimentation technique⁵. In all cases the cell suspensions were incubated at 37 °C for 1 h to reduce the level of cytophilically bound immunoglobulin. The lymphocytes were suspended in 1 ml of Eagle's MEM (Glasgow modification) supplemented with 10% heat inactivated FCS (MEM-F) to a cell concentration of 2×10^6 ml⁻¹. An appropriate volume (0.03 or 0.06 ml) of anti-allotype serum was added and the cells cultured for 24 h at 37 °C in 10% CO₂ in air. The cells were spun down, residual antiserum removed and the cells washed once in warm MEM-F, before reculturing in antibody-free MEM-F for 0, 1, 24 and 48 h. At each time period the cells were washed three times (finally in PBS) and fixed in 2% paraformaldehyde solution⁶. At the end of the experiment the fixed cells were tested for the presence of allotypic determinants characteristic of the membrane Ig and of the attached antibody by a mixed antiglobulin test (see Table 1). The allotypic determinants are known to survive the fixation procedure⁷ whereas antibody activity is destroyed. A rosette assay was chosen because it has the unique advantage of detecting surface Ig and excluding intracellular Ig.

The fate of the anti-allotype antibody and of the membrane Ig receptors is shown in Table 1. Anti-allotype antibody was readily detectable on lymphocyte membranes immediately after 24 h of incubation with antiserum and persisted on approximately the same number of cells for a further 2 d. A significant number of the cells bearing antibody appeared to be blast cells (see parentheses, Table 1). Little of this antibody seems to be taken up cytophilically on Fc receptors, since similar results were obtained with an F(ab')₂ anti-allotype preparation. The rapid disappearance from the cell membrane of any Ig taken up from a normal b6 serum provides further evidence that the persistence of antibody is unlikely to be due to cytophilic binding (Table 2). However, as demonstrated using normal b6 serum, some cytophilic uptake of Ig does take place during the initial 24 h incubation, which appears to be greatly reduced 1 h after washing and effectively lost by 24 h in culture. This cytophilic uptake appears to be entirely by the Fc receptor since it did not occur with the F(ab')₂ preparation.

Immediately after the initial incubation the washed lymphocytes exhibited high levels of bound antibody, but

low levels of membrane Ig. After 24 h incubation most of the cells appeared to recover their membrane Ig which was maintained over a further 24 h in culture. The proportion of blast cells expressing membrane Ig was approximately the same as that on which the anti-allotype antibody was present.

The observation that complexes appear in the supernatants of cultured mouse lymphocytes after only 2 h of incubation with anti-Ig⁸ prompted an experiment to investigate the possible nonspecific uptake by lymphocytes of "shed" membrane Ig-anti-Ig complexes. For this experiment b9b9 lymphocytes were cultured in a supernate (presumed to contain b4 m-Ig-anti-b4 complexes) obtained after a 3-h incubation of b4 cells with antibody. The cells were fixed after 1, 18 and 42 h and rosetting performed. Less than 1% (3 in 500 cells) of the cells formed rosettes (and these seemed to be macrophages), indicating that cytophilic up-

Table 1 Anti-Ig antibody and membrane Ig on cultured lymphocytes after prior exposure to anti-Ig serum

Experiment no.	Anti-allotype serum present during first 24-h culture (final concentration)*	Reculture time (h)	Lymphocytes (%) bearing membrane-Ig or antibody-Ig	
			Membrane-Ig	Ab-Ig
1	None (control)	0	50	—
		24	70	—
		48	70	—
	Anti-b4 (b5b6) (1 in 30)	0	7	46
		1	8	30
		24	53 (3)	32 (2)
	Anti-b4 (b5b6) (1 in 15)	48	48 (17)	53 (17)
		0	17	53
		1	17	38
		24	64 (21)	45 (17)
2	None (control)	48	65 (39)	69 (31)
		0	37	—
		24	51	—
	Anti-b4 (b5b6) F(ab') ₂ preparation (100 µg ml ⁻¹)	48	60	—
		0	2	27
		1	6	30
		24	35 (9)	37 (12)
		48	34 (12)	44 (18)
3	None (control)	0	44	—
		24	57	—
		48	61	—
	†Anti-b5 (b6b6) (1 in 15)	0	1	35
		1	0	24
		24	28	23
		48	39	29
4	None (control)	0	46	—
		24	47	—
	Anti-b6 (b4b4) (1 in 30)	0	39 ²⁶ (c) 13 (r)	46
		24	34 ¹⁰ (c) 24 (r)	28 (2)
		48	51 ⁶ (c) 45 (r)	49 (14)

Briefly the method for the mixed anti-globulin rosette assay⁶ was as follows. The fixed lymphocytes were mixed with a 1/50 dilution of anti-allotype serum (either anti-b4 or anti-b6) for 30 min at room temperature and washed. SRBC (5% suspension) sensitised with a 1/60 dilution of either b4 or b6 rabbit anti-SRBC Fab' preparation for 30 mins at 37 °C and washed. Sensitised SRBC (0.06 ml of a 1% suspension) and the sensitised lymphocytes (0.06 ml of 2×10^6 ml⁻¹) were mixed and centrifuged at 200g for 2 min. The cell pellet was gently resuspended and Toluidine blue added (0.03 ml of a 0.2% solution). Lymphocytes binding four or more RBC were counted as positive and expressed in the table as the mean of duplicate tests. Appropriate specificity controls were included to test for the non-specific uptake of the sensitising antibody by paraformaldehyde fixed cells. No detectable uptake of an irrelevant antiserum (that is anti-b6 on b4b4 cells) was ever observed.

*All anti-allotype sera were used at optimum concentrations for lymphocyte stimulation. Lymphocytes of allotype b4b4 were used in experiment 1, 2 and 3, and of b6b6 in experiment 4.

†Anti-b5 showed cross reactivity with b4 Ig determinants.

Figures in parentheses denote the percentage of blast cells rosetting. (c) = caps; (r) = rings.

Table 2 Nonspecific uptake of normal serum Ig and of "shed" membrane Ig-anti-Ig complexes by lymphocytes

Experiment no.	Initial culture composition and duration	Reculture time (h)	Lymphocytes (%) positive for Ig of extrinsic type
1	b4b4 lymphocytes cultured 24 h with b6b6 normal serum (1 in 15)	0	21
		1	6
		24	1
		48	3
2	b9b9 lymphocytes cultured with supernate from b4 m-Ig-anti-b4 culture	1	<1
		18	<1
		42	<1
			<1

Rosetting was performed as described in the legend to Table 1. For experiment 2, approximately 12×10^6 lymphocytes (Ficoll-trisil purified) were cultured at $6 \times 10^6 \text{ ml}^{-1}$ in MEM-F with 0.06 ml anti-b4 (b5b6) serum for 30 min at 4 °C. The cells were washed once in the cold, then cultured at 37 °C for 3 h in MEM-F. The supernates (0.6 ml) were then transferred to 2×10^6 b9b9 lymphocytes. After 1, 18 and 42 h at 37 °C, the cells were washed and fixed in paraformaldehyde and rosetting performed.

take of complexed antibody is not a significant source of cells expressing allotypic antibody determinants.

The experiments described demonstrate that some anti-allotype antibody persists for at least 48 h in culture after it has become fixed to the cell surface. This is in apparent conflict with the efficient "capping" of fluorescein-labelled antibody which has been shown to occur in this system⁹. There is also evidence in the experiments described here for the "capping" of antibody in the form of "capped rosettes". The explanation of these findings is that a part, perhaps a large part, of the antibody attached to membrane Ig is removed relatively rapidly by "capping" but the remainder persists for very long periods. Alternatively, antibody may persist as a result of binding to resynthesised membrane Ig. The fact that antibody persists on the surface well beyond 24 h is compatible with the induction of activation by anti-allotype serum. These results are consistent with findings for the fate of concanavalin A (con A) and phytohaemagglutinin (PHA) in lymphocyte cultures where about 70% of the surface-bound lectin has been shown to be rapidly removed, whereas the remainder is retained for longer periods¹⁰.

Lymphocytes do not seem to be unique in the mode of retention of surface-fixed substances. Other workers have demonstrated the persistence of KLH on membranes of macrophages¹¹, antigen-antibody complexes on dendritic cells¹² and DNP-D-GL copolymers on the surface of lymphocytes¹³ for long periods. ALG has also been shown to persist in micro patches on mouse lymphocytes for several hours¹⁴, a fact which is particularly interesting in view of the known lymphocyte-stimulating activity of these reagents¹⁵.

In conclusion, it has been demonstrated that antibody to membrane Ig persists on lymphocyte membranes for up to 3 d in culture. During this period membrane Ig is being resynthesised and blast transformation is taking place.

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Immunodepression, high IgM levels and evasion of the immune response in murine trypanosomiasis

THERE is currently much interest in the devices evolved by parasites which enable them to evade the immune responses mounted by their hosts¹. One such device is the phenomenon of antigenic variation associated with the salivarian trypanosome infections of man and animals in Africa^{2,3}. Typically these infections show successive waves of blood parasitaemia. Each succeeding trypanosome population is characterised by a novel variant-specific glycoprotein antigen present in the parasite surface coat and not reactive with antibodies induced against preceding variants^{4,5}. Antigenic variation has a clear role in the evasion of host immunity and leads to chronic and usually fatal infections. More recently we have become aware of a possible additional component of the evasion mechanism, generalised immunodepression.

Depressed responses to heterologous antigens have been shown in experimental lymphomagenic virus infections^{6,7}, in malaria^{8,9}, and in trypanosome infections^{10,11}. Explanations of the immunodepression in trypanosomiasis are complicated by the concomitant high levels of IgM immunoglobulins, much of

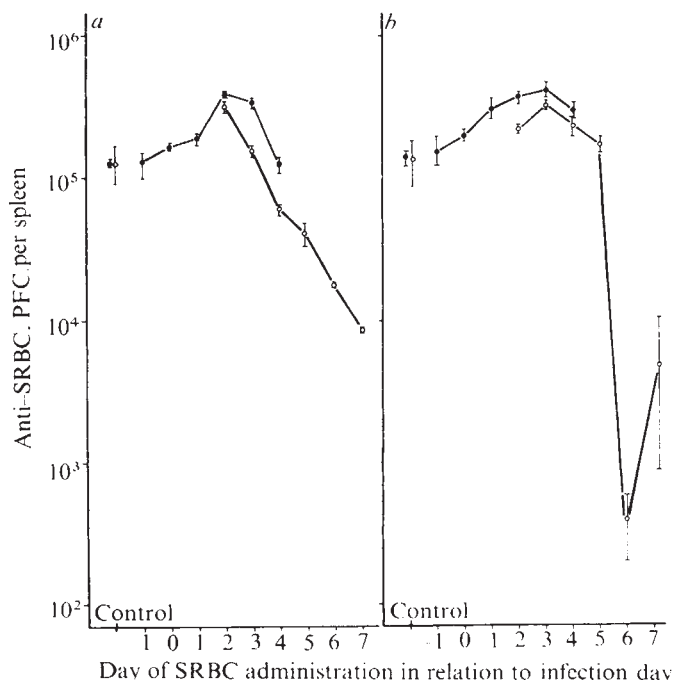


Fig. 1 Altered responsiveness of *T. b. brucei*-infected mice to SRBC. Numbers of PFC in female BALB/c mice given 1×10^9 SRBC intraperitoneally various days before or after infection with *T. b. brucei* S42, 1,000 organisms given intraperitoneally. Each point is the arithmetic mean (± 1 s.e.) of the PFCs per spleen of five mice, and the points represent two overlapping series, series 1 (●), series 2 (○). PFC assayed by the slide method²⁴. a, Direct (IgM) PFC; b, indirect PFC developed using a rabbit anti-mouse specific IgG_{2a} antiserum titrated and used at a concentration such that the inhibitory constant (KI) and developing constant (KD) = 1.0 (ref. 25).

which appears not to be parasite specific¹²⁻¹⁵. Following a detailed study of the plaque-forming cell (PFC) response to sheep red blood cells (SRBC), and to other heterologous erythrocytes and antigens, in mice infected with *Trypanosoma brucei brucei*, we are able to suggest a possible link between high IgM levels and immunodepression. We further suggest that less than optimal anti-parasite responses result from the immunodepression which thus assists the parasite to evade host immunity.

Figure 1 summarises the data of a number of experiments in which the time intervals between infection and SRBC immunisation were varied. Experimental details are given in the figure and table legends. When SRBC were injected 1-4 d after infection there was enhanced responsiveness, with both IgM and IgG-PFC being 2-3 times those of the uninfected immunised controls. But when SRBC were given 5 or more days after infection, the PFC responses were diminished; at 7 d both IgM and IgG PFC were equal only to background values. This profound depression persisted for the remainder of the infection. Clearly the effect of the infection on the PFC response depends on the temporal relationship between the day of infection and the antigen presentation; responses early in infection may be enhanced whereas later responses are depressed. These findings are broadly similar to the enhancement or suppression of haemolysin production in mice treated with bacterial lipopolysaccharide (LPS)¹⁶. The response was enhanced if LPS was given at the same time or shortly after SRBC, but was largely inhibited if LPS was given 1 or 2 d before the antigen.

We also observed a progressive increase in "background" IgM PFC to SRBC in infected, non-immunised mice. The time course of this background increase is shown in Fig. 2 in relation to the blood parasitaemia. By days 3-4 of infection a pronounced rise is seen which reaches plateau levels by about day 9. Numerous observations indicate that the plateau level of IgM PFC is usually 20-30 times that of uninfected control mice. We next investigated the background PFC of infected mice to two other heterologous erythrocytes, and to three other antigens coated on to SRBCs (Table 1). The values in Table 1 are mainly expressed as PFC per spleen because our interest centres on the total Ig production of the animal. As, however, splenomegaly develops in murine trypanosomiasis, we also express PFC per 10^6 spleen cells in selected instances (Table 1b). In all cases, background IgM PFC were significantly increased,

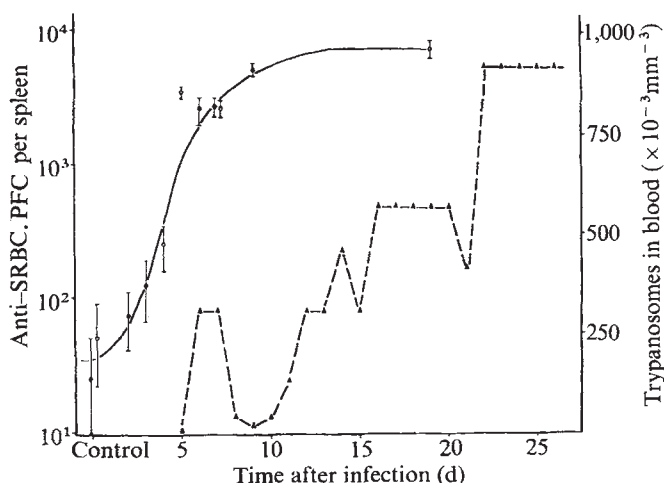


Fig. 2 Rise in SRBC background IgM PFC in *T. b. brucei*-infected mice in relation to blood parasitaemia. Male BALB/c mice were infected with 1,000 *T. b. brucei* S42 intraperitoneally. Each point is the arithmetic mean (± 1 s.e.) of the PFC per spleen of four or five mice. The points represent direct (IgM) PFC in two overlapping series, series 1 (●), series 2 (○), all groups within a series being assayed on the same day. Each point on the parasitaemia curve (▲—▲) represents the mean blood parasitaemia count of 10 mice.

particularly so with the TNP hapten. Because of the number and variety of antigens tested, it seems not unreasonable to assume that the background IgM responses to all antigens recognisable by B cells are increased in trypanosome-infected mice, and that the trypanosome infection therefore induces polyclonal B-cell activation. Indeed, Coutinho and Moller¹⁷ have argued that a marked increase in background IgM PFC to a highly hapten-substituted target cell, such as our TNP-coated SRBCs, is in itself a good measure of polyclonal B-cell activation. They state that such rises, although not specific, reflect increases in the background responses to a variety of cross-reacting determinants. Again a parallel may be drawn between the polyclonal activation of trypanosome infections and that produced by LPS¹⁸. The secreted polyclonal products would then account for the increased Ig levels seen in the infection; by day 14 serum IgM levels had increased 8-16-fold

Table 1 Increase in number of background PFC to a variety of antigens in mice infected with *T. b. brucei*

a PFC per spleen		Day of infection			
Antigens		Controls	8	10	19
SRBC		215 $\pm$ 58	5,380 $\pm$ 779	—	—
SIH	Direct	301 $\pm$ 116	1,128 $\pm$ 230	—	—
CGG	PFC	216 $\pm$ 52	2,652 $\pm$ 700	—	—
TNP		964 $\pm$ 146	31,302 $\pm$ 2,289	—	—
SRBC	Direct	281 $\pm$ 107	—	—	7,000 $\pm$ 1,036
	Indirect	0	—	—	2,391 $\pm$ 598
HRBC	Direct	63 $\pm$ 63	—	—	2,000 $\pm$ 289
	Indirect	0	—	—	756 $\pm$ 222
DRBC	Direct	94 $\pm$ 31	—	—	3,313 $\pm$ 542
	Indirect	0	—	—	1,173 $\pm$ 480
b PFC per spleen and per 10^6 spleen cells					
PFC per spleen					
SRBC	Direct	200 $\pm$ 55	—	11,080 $\pm$ 1,693	—
CGG	PFC	250	—	3,523 $\pm$ 970	—
PFC per 10^6 spleen cells					
SRBC	Direct	4 $\pm$ 0.4	—	29 $\pm$ 5	—
CGG	PFC	4 $\pm$ 0.24	—	9 $\pm$ 2.2	—

Each PFC is the arithmetic mean (± 1 s.e.) of 4-5 mice. Antigens: SRBC, HRBC, DRBC; sheep, horse and donkey erythrocytes: SIH; SRBCs coated with type III pneumococcal capsular polysaccharide using broth culture supernatant²⁶; CGG; SRBC coated with chicken gamma globulin by incubating equal volumes of 10% SRBC and chicken anti-SRBC antiserum ($\frac{1}{2}$ minimum agglutinating titre) at 37 °C for 30 min. and washing three times: TNP; trinitrophenol determinant coated on to SRBC using TNBS²⁷. The values for SIH, CGG and TNP were calculated according to the formula: PFC.Ag = PFC.Ag SRBC - (PFC.SRBC $\times$ CF). The correction factor (CF) is a measure of the efficiency of lysis of antigen-coated SRBC by anti-SRBC-PFC, and thus reflects the degree of antigen coating. For example in the first series, CF values for SIH, CGG and TNP were 0.46, 0.48 and 0.63. These ratios were established on the day of assay using the same batches of antigen-coated SRBC as in the experimental groups, and were calculated from the geometric means of 10 duplicates using $\log(x + 1)$ transformation. The assays were performed on the pooled spleen suspensions from three BALB/c mice given 1×10^8 SRBC intraperitoneally 5 d previously.

and IgG_{2a} had increased four- to fivefold in these *T. b. brucei*-infected mice (Hudson and Byner, unpublished observations). Table 1 does show increases in background IgG PFC to sheep, horse and donkey RBC, but these increases vary widely from experiment to experiment and never approach the increases seen in background IgM PFC. Polyclonal B-cell activation has previously been suggested to occur in malaria and trypanosomiasis^{19,20}, but hitherto mitogenic activity of these parasites has only been demonstrated *in vitro*^{21,22}.

We believe that the polyclonal activation of B cells in the presence of a continuous trypanosome infection is likely to result in a progressive depletion of antigen-reactive B lymphocytes as these are activated to change into secretor cells, perhaps with little accompanying proliferation. In this way the immunodepression occurring later in the infection may be explained by the depletion of B cells capable of recognising the introduced antigen. Diamantstein and his colleagues²³ have shown suppression of primary responses *in vivo* to SRBC by a number of B-cell mitogens. They believe that when B cells react with a mitogen in the absence of a particular antigen, they temporarily lose their capacity to respond to that antigen. The longer term immunodepression associated with murine trypanosomiasis might then result from the continuous exposure of B cells to trypanosomes and their products.

Finally, if both activation and immunodepression are polyclonal it would be expected that the antibody responses to later-appearing trypanosome antigenic variants would also be impaired. The pattern of infection with S42 *T. b. brucei* (Fig. 2) lends some support to this view. The infection shows waves of parasitaemia which have been shown to be antigenically distinct (K.M.H., C.B., and A. E. R. Taylor, unpublished). The first wave subsides around days 8–9, due to the production of variant specific antibodies. The second major wave, in spite of some minor fluctuations, never subsides to the same degree as the first wave but continues to rise until the death of the mouse. This may perhaps indicate that the production of variant specific antibodies is depressed at this stage of the infection. We are at present attempting to obtain direct evidence on this point and are also investigating a series of more chronic infections. Meanwhile it is a reasonable working hypothesis that high IgM levels, immunodepression of responses to heterologous antigens, and the less than fully effective anti-trypanosome responses are linked, and are brought about by the trypanosomes either directly or indirectly activating the host B cells polyclonally.

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Absence of intestinal mast cell response in congenitally athymic mice during *Trichinella spiralis* infection

DURING the intestinal phase of nematode infections in laboratory rodents a variety of cell types is found in the gut mucosa, including mast cells and globule leukocytes^{1–4}. After stimulation by the nematodes present either in the intestinal lumen or penetrated into the gut mucosa, the number of intestinal mast cells increases^{1–4}. Next, mast cells invade the epithelial lining of the villi, discharge secretory products, including histamine, and change into the intra-epithelial globule leukocytes^{1–3}. Release of mast cell secretory products is mediated by IgE antibody⁶. No conclusive evidence has as yet been presented, that the secretory products have a role in the expulsion of the worms.

In earlier studies⁴ the thymus dependence of both intestinal mast cell production and globule leukocyte formation was studied in *Trichinella spiralis* infected rats with and without anti-thymocyte serum (ATS) treatment. ATS treatment did not influence mast cell production, but had an adverse effect on mast cell degranulation which was attributed to a possible decrease in IgE level in the ATS-treated rats. Since in ATS-treated animals a residual pool of T cells can be expected as well as an intact thymus, we repeated these experiments in congenitally athymic (nude) mice and their heterozygous thymus-bearing (+/nu) littermates. In +/nu mice *T. spiralis* adult worms are expelled from the intestinal tract within 10 d after infection, whereas in nude (nu/nu) mice worms are present until at least 50 d after infection^{7,8}, indicating T-cell dependence of host protection against a *T. spiralis* infection.

Male SPF B10LP nude (nu/nu) mice, 6 weeks of age, were obtained from the Central Laboratory for the Breeding of Laboratory Animals TNO, Zeist, The Netherlands, where the mice were maintained by conventional back crossing with strain B10LP. Comparisons were made with age-matched mice heterozygous for the nude gene (+/nu). At an age of 8 weeks the mice were orally infected with 100 *T. spiralis* larvae each, using a syringe. Animals were autopsied from 3 to 16 d after infection. The presence of intestinal mast cells and globule leukocytes was studied in a 10-cm portion of the proximal jejunum with a swiss-roll technique⁹. Tissues were fixed in a special fixative, adopted after preliminary studies, since intestinal mast cells differ in their staining properties from tissue mast cells elsewhere in the body and the fixative proposed for rat intestinal mast cells¹⁰ proved inadequate. The fixative was composed of a mixture of 0.8% formalin buffered with phosphate-buffered

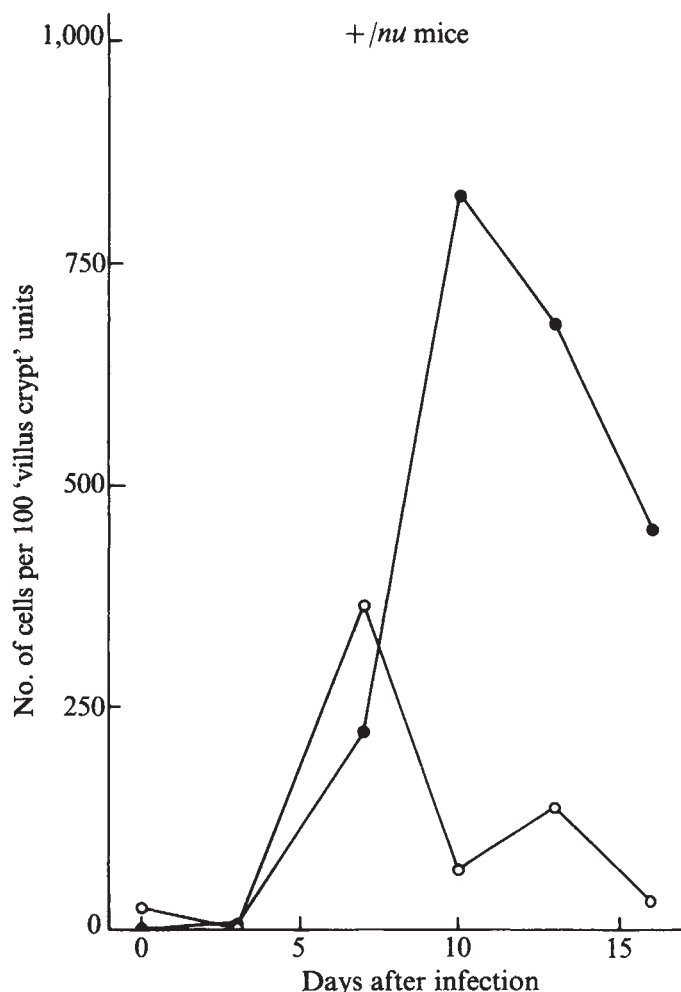


Fig. 1 Mean number of intestinal mast cells and globule leukocytes in a 10-cm portion of the proximal jejunum (expressed per 100 "villus crypt" units) in groups of 4–5 $+/\nu$ mice infected orally with 100 *T. spiralis* larvae each. Statistical analysis (Student's *t* test). Mast cells: day 3 against day 0, $P < 0.001$; day 7 against day 0, $P < 0.01$; other days after infection against day 0, not significant. Globule leukocytes: day 3 against day 0, not significant; day 7 against day 0, $P < 0.001$; day 10 against day 0, $P < 0.001$; day 13 against day 0, $P < 0.01$; day 16 against day 0, $P < 0.001$. ○, Mast cells; ●, globule leukocytes.

saline (0.01 M; pH 7.2) at neutral pH and 4% acetic acid. After overnight fixation at 45 °C tissues were dehydrated and embedded in paraplast according to conventional procedures. Sections (5 μ m thick) were prepared and stained with toluidine blue for 20 min at room temperature. Toluidine blue (0.5%) was dissolved in 20% ethanol. After filtration the solution was used for staining. After staining the sections were washed, dehydrated and mounted with a conventional mounting medium.

Cells present in the intestinal mucosa were counted in the whole swiss-roll and their number expressed per 100 "villus-crypt" units. A "villus-crypt" unit represents a portion of gut mucosa, lying between two gland crypts and the lamina propria of the villus above¹¹. Non-*T. spiralis*-infected animals served as controls (day 0 in Figs 1 and 2).

The results of the mast cell and globule leukocyte counts in the $+/\nu$ mice are shown in Fig. 1, the corresponding counts for the ν/ν mice in Fig. 2.

In $+/\nu$ mice, the number of intestinal mast cells decreased at day 3 after infection and showed a rise at day 7, after which a gradual decline was observed. The number of globule leukocytes increased noticeably from day 7 to day 10 after infection; at days 13 and 16 a decline was seen. In ν/ν mice, mast cells and globule leukocytes were present at day 0, although the number was much lower

than that found in $+/\nu$ mice. During the course of the infection no change in number of either cell type was observed.

The intestinal mast cell response in $+/\nu$ mice corresponds with that described for rats infected with *T. spiralis*⁴ or *Nippostrongylus brasiliensis*¹. The initial decrease in number of mast cells is attributed to toxic metabolites secreted by the parasite¹². The subsequent increase in number of intestinal mast cells has been tentatively attributed to proliferation of precursor cells of lymphoid origin¹³. The increase of globule leukocytes is in keeping with the intestinal mast cell degranulation theory.

The fact that in non-infected ν/ν mice only occasional intestinal mast cells and globule leukocytes were found is in contrast to the abundance of tissue mast cells elsewhere in the body of these athymic mice, as observed by us and others¹⁴. Furthermore, the continuous presence of the parasites⁴ did not cause an increase in either intestinal mast cell or globule leukocyte numbers.

An explanation for these findings could possibly be found in the as yet unknown origin of the mast cell. Various authors have suggested that mast cell formation may be

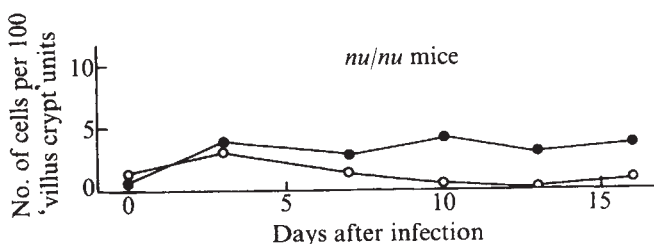


Fig. 2 Mean number of intestinal mast cells and globule leukocytes in a 10-cm portion of the proximal jejunum (expressed per 100 "villus crypt" units) in groups of 4–5 ν/ν mice infected orally with 100 *T. spiralis* larvae each. Statistical analysis (Student's *t* test). Mast cells: all days after infection against day 0, not significant. Globule leukocytes: all days after infection against day 0, not significant. ○, Mast cells; ●, globule leukocytes.

thymus dependent^{15–18}. Burnet¹⁶ speculated that mast cells and basophil leukocytes, or some subpopulation of such cells, are post-mitotic derivatives of thymus-derived (T) lymphocytes. On the other hand, thymic hormones can possibly also have a role in the differentiation of mast cells¹⁸.

To examine this possibility we performed reconstitution experiments. Thymus transplants of 6-week-old male donor B10LP $+/\nu$ mice were grafted subcutaneously in a group of 9 male B10LP ν/ν mice, 6 weeks of age (two thymuses per recipient). The animals were autopsied at an age of 12 weeks. A group of 10 male B10LP $+/\nu$ mice and a group of seven non-reconstituted male B10LP ν/ν mice, served as controls. Animals of both groups were 12 weeks old. The number of intestinal mast cells was counted in a 10-cm portion of the jejunum. The number of intestinal mast cells in the reconstituted mice was comparable (20–30 cells per 100 "villus crypt" units) to the number of cells counted in the jejunum of the $+/\nu$ mice, whereas none were seen in the non-reconstituted ν/ν mice which is consistent with thymus dependence of the formation of intestinal mast cells.

In all published studies results are based on tissue mast cells. In the athymic mouse tissue (non-intestinal) mast cells seem to be as abundant as in its thymus-bearing littermates. This is not consistent with the idea that the tissue mast cell is an end-stage of the T lymphocyte. In the formation of intestinal mast cells, however, the thymus clearly has an essential role.

The conclusions of the present experiments are therefore: (1) no intestinal mast cell response accompanies a *T. spiralis*

infection in congenitally athymic (nude) mice, and (2) intestinal mast cells represent a separate population of mast cells, which is "T dependent".

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Virus-like particles and GB agent hepatitis

ALTHOUGH the MS-1¹ and CR326² strains of human hepatitis A virus have been well characterised morphologically and shown to be indistinguishable, controversy has plagued characterisation of the GB agent of hepatitis³. Presumably derived from a surgeon with acute hepatitis, the GB agent has been transmitted serially in marmoset monkeys³ but has been shown to be unrelated serologically to any known human hepatitis virus⁴. Recently, Almeida *et al.*⁵ reported the detection after prolonged search of 20-22-nm particles aggregated by endogenous antibody in a pool of marmoset sera (pool H205, GB pass 11) known to contain the GB agent. These antibody-coated particles resembled parvoviruses morphologically, appeared in "empty", "full", and fragmented crescent-shaped forms, and were undetectable in normal marmoset serum. On the basis of previous filtration data from Deinhardt *et al.*⁶ which suggest that the GB agent is approximately 20 nm in diameter and the previous finding by others that anti-complementary activity—thought to represent the presence of circulating antigen-antibody complexes—occurs during acute viral hepatitis, Almeida *et al.* suggested that the virus-like particle they have detected is the GB agent⁵.

Using a different approach in an attempt to detect the GB agent, we examined by immune electron microscopy homogenates of liver obtained during the height of acute GB infection from individual *Saguinus mystax* and *S. nigricollis* marmosets that had been inoculated with GB agent pools provided by Deinhardt. The same approach had been used successfully by Provost *et al.*² to identify the CR326 strain of human hepatitis A virus in marmoset liver. The homogenate of one liver obtained from a *S. mystax* marmoset (No. 49) (inoculated with the GB H205 pool) when serum isocitrate dehydrogenase activity was 4,680 IU (>4 times normal) was clarified and banded in a caesium chloride (CsCl) gradient (1.1-1.6 g cm⁻³). Each gradient fraction was incubated with convalescent serum from a marmoset (*S. mystax* No. 50) convalescent from hepatitis induced by the H205 pool of GB agent and examined by immune electron microscopy¹. As shown in the figure, aggregates of approximately 34-36-nm diameter particles resembling viruses with icosahedral symmetry were

detected at a buoyant density of 1.4 g cm⁻³. Both "empty" and "full" particles were observed, but empty particles were more abundant. In limited serological studies performed under code, when these particles were incubated with pre-inoculation serum from marmoset No. 50, antibody-coated aggregates were also detected, but no antibody was seen when the particle-containing gradient fraction was incubated with saline. In addition, no antibody to this particle could be detected in the convalescent serum of the surgeon from whom the GB agent was isolated.

The diameter of these particles corresponds closely to the size deduced by Parks and Melnick⁷ for the GB agent from their filtration data; however, the failure of this virus-like antigen to distinguish under code between pre-inoculation and convalescent GB serum raised questions about its relationship to the GB agent. To pursue these findings, we examined CsCl gradients from homogenates of livers obtained from five *S. mystax* marmosets with hepatitis A virus infection, from one normal *S. mystax* marmoset, from one *S. mystax* marmoset convalescent from GB infection, and from two *S. nigricollis* marmosets acutely infected with a different GB agent pool (H118).

Virus-like particles indistinguishable from those seen in

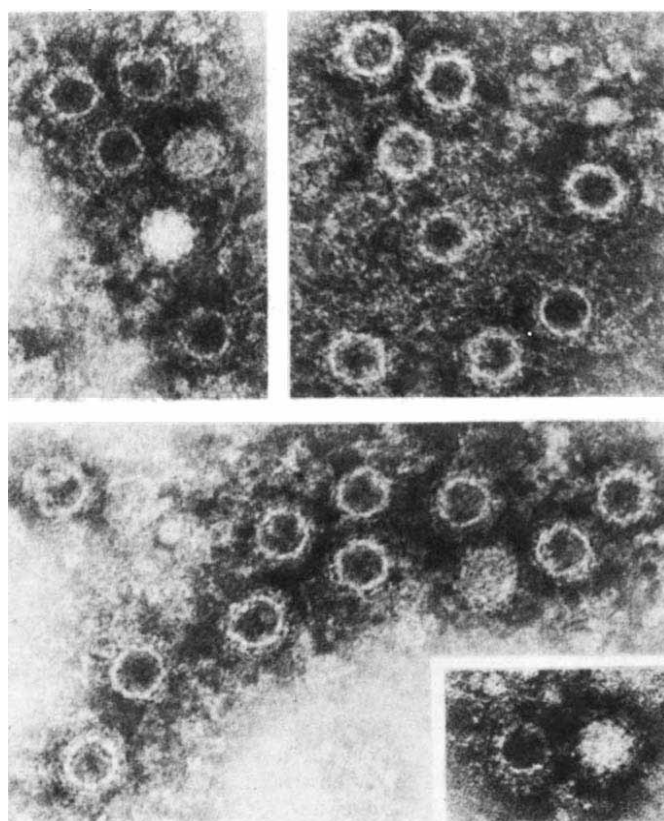


Fig. 1 Examples of aggregates of antibody-coated 34-36-nm particles detected in a liver homogenate from *S. mystax* marmoset No. 49 acutely infected with the GB agent. The liver was frozen at -70 °C immediately after the death of the marmoset. A 20% homogenate of the liver was made in distilled water after mincing with fine scissors and grinding with mortar and pestle. After the homogenate was clarified to remove large debris (1,000g for 1 h at 4 °C), 4 ml of supernatant fluid was layered on a 9-ml discontinuous preformed 6-step CsCl gradient (1.1-1.6 g cm⁻³) prepared in a cellulose nitrate tube for a Beckman SW 40 rotor (Beckman Instruments). The gradient was centrifuged in a Beckman L2-65B ultracentrifuge for 18 h at 35,000 r.p.m. (4 °C) and collected from the bottom in fractions of approximately 1 ml each. Each fraction was incubated with convalescent serum from *S. mystax* marmoset No. 50, which was convalescent from GB infection, and examined by immune electron microscopy as described¹. (Original magnification 194,400. 2% phosphotungstic acid stain.)

the first GB-infected liver were found in the two livers from marmosets acutely infected with the GB agent but in neither of the livers from the normal or GB-convalescent marmosets nor in livers from marmosets with hepatitis A virus infection (Table 1). Unfortunately, the quantity of antigenic material extracted was insufficient to allow more extensive serological evaluation, and the particles seemed to be relatively unstable after extraction.

Correlation of the size of these particles with size determinations performed by filtration of the GB agent⁷ and the specificity of their detection in GB-infected livers suggest that they may be the GB agent. If this is true, the presence of antibody in preinoculation serum is difficult to explain, unless (1) GB-related illness represents reinfection with a virus serologically related to a previously experienced virus, or (2) the GB agent is a latent virus of marmosets to which antibody is produced but which can become reactivated during periods of stress, like herpesviruses of man. Such an hypothesis agrees with the isolation of a GB-like agent from uninfected marmosets, as reported by Parks and Melnick⁸. Indeed, even if the 20–22-nm particle described by Almeida *et al.* is the GB agent, the fact that antibody to it exists in the infectious inoculum suggests an unusual immunological relationship between virus and host.

Table 1 Virus-like particles in the livers of marmosets

Category of marmoset	No. with 34–36-nm particles in liver/No. tested
GB agent-infected	
Acute	3/3
Convalescent	0/1
Hepatitis A virus-infected (acute)	0/5*
Normal	0/1

*27-nm hepatitis A antigen particles were detected in three of these.

Another discrepancy between earlier studies of the GB agent and both Almeida's and this report concerns the buoyant density of the particles. Original banding studies by Holmes *et al.*⁴ revealed a peak infectivity at a density of 1.18–1.23 g cm⁻³ for both the GB agent and the MS-1 hepatitis A virus strain. The particles identified by us have a density of approximately 1.4 g cm⁻³, and if Almeida's particles are parvoviruses or even parvovirus-like, a density of 1.4 g cm⁻³ or greater is to be anticipated. Although hepatitis A virus is known to be infectious at densities ranging from 1.15 to 1.41 g cm⁻³, the main density is thought to be approximately 1.34 g cm⁻³ (ref. 2); infectivity at densities <1.2 g cm⁻³ probably represents virions bound to lipid-containing contaminants. Conceivably the original biophysical data of Holmes *et al.* for both the GB agent and hepatitis A virus were derived with such lipid-associated material and do not represent the true density suggested by more recent studies.

If indeed, as Almeida's study suggests, the GB agent is parvovirus-like, the lipid solvent sensitivity and the instability of the GB agent reported by both Deinhardt's and Melnick's groups^{7,9} are in contrast to the marked heat stability and resistance to lipid solvents that characterise the parvoviruses¹⁰.

Clearly, data to support the candidacy of the 20–22-nm particle or the 34–36-nm particle for the elusive GB agent are incomplete. Additional studies are required to determine whether (1) either of these particles is related to GB infection, (2) an as yet unidentified particle is the GB agent, (3) GB is of human or marmoset origin, and (4) the parvovirus-like particles are related to those recently detected in human serum¹¹. Morphologically similar 22-nm particles are ubiquitous in faeces, and, although once

thought related to viral gastroenteritis, are no longer considered to be associated with any known human disease¹². Moreover, since Almeida *et al.* looked at a pool of sera, antibody from one serum might have aggregated virus in another. It will therefore be necessary to examine individual marmoset sera obtained during GB infection to establish the presence of circulating immune complexes. Until a virus-like particle is identified which can be serologically related to GB illness or can be aetiologically associated with infection by other means, morphology of the GB agent will remain a matter of conjecture.

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Avian cell transformation and the expression of avian sarcoma virus-specific tumour antigens

MALIGNANT cell transformation is often accompanied by the appearance of new cell-surface antigens^{1–3} of four classifications (defined in ref. 3): (1) embryonic antigens (EA) that are also present on normal embryonic cells; (2) differentiation antigens (DA) so called because they are also expressed on certain normal syngeneic or allogeneic cells; (3) virus structural antigens (VSA) inserted in the surface membrane of infected cells which may or may not be transformed, and (4) tumour-specific transplantation antigens (TSTA), antigens associated with tumour rejection.

Here we ask the questions first, does transformation of avian cells by agents other than avian sarcoma viruses (ASV) induce a tumour-specific surface antigen (TSSA) hitherto recognised as avian oncornavirus specific; and second, does the additional genetic information supplied by a superinfecting avian leukosis virus (ALV) or ASV lead to TSSA expression? Our experiments suggest that transformation by ASV is necessary for the induction of this antigen.

The operationally defined TSTA have received widespread attention in the past. Appropriately immunised animals will reject a subsequent challenge by viable syngeneic tumour cells because of their anti-TSTA immunity.

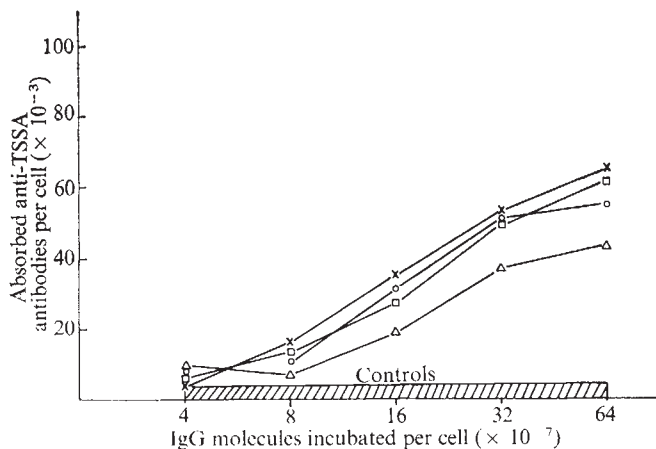


Fig. 1 Specific absorption of anti-TSSA immunoglobulins G to quail cells infected by avian leukosis sarcoma viruses and/or Kirsten sarcoma or simian sarcoma associated viruses. Cells growing in 16-mm Linbro 96 CV-TC tissue culture plates were counted and fixed at subconfluency (about 10^6 cells per culture) and incubated in duplicates with exactly equal amounts ($16 \mu\text{g}$ or dilutions thereof) of ^{125}I -IgG from normal or anti-TSSA (No. 62) chicken sera as described previously^{8,9}. Incubation was stopped by aspiration of the IgG dilutions and 4 washes of 5 min each with PBS. The absorbed antibodies were eluted from the cells with pH 1.2 KCl-HCl buffer and their radioactivity was assessed in a well-type scintillation counter. The number of specifically absorbed antibodies was calculated from the specific radioactivity of the anti-TSSA IgG-preparations minus background absorption of normal IgG (usually less than 20%). Quail cells were infected by the following virus strains: $\times$, Prague A or B77-C avian sarcoma virus strains; $\square$, Rous (-) sarcoma virus; $\circ$, 23.16 = a Rous(-) transformed quail cell line; Δ , KiSV(SSAV) plus Prague A-ASV. Uninfected cells (controls) or cells infected by the following virus strains showed no specific absorption: RAV-1, RAV-49, KiSV (SSAV), KiSV(SSAV) + RAV-1, KiSV(SSAV) + RAV-49. All data are taken from one characteristic experiment.

Tumours induced both by chemicals and by viruses express TSTA of widely varying antigenicity and immunogenicity. Whereas in the case of chemical tumours TSTA by necessity must be cell coded, their origin in virus tumours is not known. Virus-induced TSTA, which are not incorporated into virus particles, are virus-group specific. Only virus strains belonging to the same tumour virus group will induce cross-reacting TSAs.

In the avian oncornavirus system, a TSSA has been detected which seems invariably to be expressed on the cells of various species transformed by avian leukosis

sarcoma virus strains (reviewed in ref. 4). It is a rejection-inducing transplantation-type neoantigen (TSTA). Its origin is also unknown. It may be a direct viral gene product or appear as a consequence of virus-activated cellular genes. Chicken embryo fibroblasts infected but not transformed by avian leukosis viruses *in vitro* do not express TSSA, but chickens infected with leukosis viruses may develop immunity to tumour cell challenge, indicating that *in vivo* these viruses can elicit the antigen⁸⁻⁷. The expression of the genes coding for TSSA may in turn depend on the cell possessing a transformed phenotype and ALV may only find the appropriate target cells to form this interaction *in vivo*.

Avian cells are generally refractory to transformation by viruses other than the avian oncornaviruses. However, xenotropic oncornaviruses from mice and other mammals are infectious for avian cells. In this respect, the simian sarcoma associated virus (SSAV) is a xenotropic virus with this capability and can be used to construct a pseudotype with the Kirsten strain of murine sarcoma virus KiSV (SSAV) which will readily infect and transform Japanese quail cells. (This virus was provided by Dr R Weiss.)

Outbred Japanese quail cells between the second and tenth passage served as targets for virus infection and transformation. Only cells from a single embryo were used in any one experiment and the normal or anti-TSSA

Table 2 Uptake of radioactive ^3H -2-deoxyglucose by virus-infected quail cells

Virus	Morphology	Uptake $\times 10^{-6}$ cells (c.p.m.)	Ratio*
Uninfected	Normal	7,800	—
ALV†	Normal	9,125	1.17
ASV†	Transformed	48,830	6.26
Rous(-)-ASV	Transformed	42,275	5.42
KiSV(SSAV)	Transformed	38,455	4.93
KiSV(SSAV)	Transformed	45,865	5.88
‡ ALV			
KiSV(SSAV)	Transformed	48,360	6.20
‡ ASV			

*Ratio of sugar uptake of virus-infected compared with uninfected cells. Around 10^6 subconfluent cells growing in 35-mm plastic dishes were washed once in PBS at room temperature before incubation with $0.5 \mu\text{Ci ml}^{-1}$ ^3H -2-deoxyglucose for 9 min. After another four washes with ice-cold PBS 2 ml cold 5% TCA was added for 5 min. Two 0.5-ml aliquots were taken for assessment of radioactivity, which was adjusted to cell number determined from duplicate cultures.

† For abbreviations see Table 1.

Table 1 Growth of virus-infected quail cells in semi-solid agar*

Virus	Cell morphology	No. of cells seeded	No. of colonies	Cloning efficiency (%)
Uninfected	Normal	10^4	0	0
		5×10^4	0	0
ALV†	Normal	10^4	0	0
		5×10^4	0	0
ASV‡	Transformed	10^4	170	1.7
		5×10^4	1,900	3.8
Rous(-)-ASV	Transformed	10^4	520	5.2
		5×10^4	>4,000	>8.0
KiSV (SSAV)	Transformed	10^4	380	3.8
		5×10^4	2,300	4.6
KiSV(SSAV)	Transformed	10^4	200	2.0
+ ALV		5×10^4	1,400	2.8
KiSV(SSAV)	Transformed	10^4	650	6.5
+ ASV		5×10^4	2,700	5.4

*The agar suspension technique was performed as described in reference¹¹.

†ALV: avian leukosis virus strains used: RAV-1, RAV-49.

‡ASV: avian sarcoma virus strains used: Prague-A, B77-C.

IgG preparations were always exhaustively absorbed with normal cells before their use in the anti-globulin absorption technique^{8,9}. In this test the difference in absorption of radioactively labelled normal and anti-TSSA IgG molecules to mildly fixed target cells is taken as measurement for the amount of TSSA-expression (see legend to Fig. 1). RAV-1 and RAV-49 are avian leukosis virus strains which infect but do not transform quail cells. In contrast, the avian sarcoma virus strains Prague-A and B77-C both infect and transform quail cells as efficiently as KiSV(SSAV). The replication-defective Rous(-) strain of ASV was included in these studies for direct comparison with KiSV, which is also replication-defective in solitary infections without helper viruses like SSAV.

Since superinfection with leukosis viruses does not lead to phenotypic alterations of the infected cells, successful ALV infection of normal or KiSV(SSAV) infected quail cells had to be demonstrated by interference tests¹⁰. Dilutions of up to 10^{-7} of 1 ml of supernatant medium from each of the RAV-1 or RAV-49 infected cultures were used to infect normal quail cells and were able to prevent subsequent superinfection by focus-forming Prague-A or B77-C sarcoma virus strains, respectively.

Table 3 Phenotype and expression of the avian oncornavirus-induced TSSA of virus-infected Japanese quail cells

Virus strain	Virus type	Morphology	Growth in semi-solid agar*	Enhanced sugar uptake†	TSSA-expression‡
Uninfected		Normal	—	—	—
RAV-1, RAV-49	ALV	Normal	—	—	—
Prague-A, B77-C	ASV	Transformed	+	+	+
Rous(-)	Replication-defective ASV	Transformed	+	+	+
KiSV(SSAV)	KiSV = replication defective MuSV	Transformed	+	+	⊖
	SSAV = leukosis-type primate oncornavirus				
KiSV(SSAV) plus ALV-strains	See above	Transformed	+	+	⊖
KiSV(SSAV) plus ASV-strains	See above	Transformed	+	+	⊕

*For details see Table 1.

†For details see Table 2.

‡For details see Fig. 1.

This high interfering titre indicated that most if not all cells had been successfully infected by ALV-strains.

Morphological transformation of quail cell cultures occurred within two passages after infection by the avian or murine sarcoma virus strains. First it had to be shown by criteria other than morphology that these cells were indeed transformed. Table 1 shows that only the sarcoma virus transformed quail fibroblasts possessed the ability to grow and form colonies in semi-solid agar¹¹. Superinfection of KiSV(SSAV) transformed cells by ALV or ASV did not alter their potential for colony formation. In contrast, uninfected or ALV-infected non-transformed cells showed no ability to grow in agar.

Another characteristic parameter of transformed cells is their enhanced rate of uptake of certain sugars^{12,13}. Again only the quail fibroblasts transformed by sarcoma virus strains expressed this property (Table 2).

Having thus defined the normal or transformed phenotype of the virus-infected cells, the specific absorption of anti-TSSA immunoglobulins to the cell surfaces was tested. As can be seen in Fig. 1, only fibroblasts infected or superinfected by avian sarcoma viruses absorbed anti-TSSA antibodies. Cells transformed by the murine sarcoma virus strain KiSV did not express TSSA even when superinfected with ALV. However, superinfection of KiSV-(SSAV) transformed quail cells with ASV strains led to TSSA-induction. All TSSA-positive cell types expressed a similar amount of TSSA, whereas the TSSA-negative cells showed practically no specific absorption.

Table 3 gives a summary of the phenotypic properties of the virus-infected cells. The encircled results point to a clear difference in TSSA expression in the cells tested.

Infection and transformation of fibroblasts by ASV leads to the induction of the avian TSSA; infection by ALV does not (reviewed in refs 2-4, and 14). TSSA-expression seems to be a consequence of ASV-transformation. All ASV-transformed cells of a variety of avian or mammalian species expressed TSSA, which is thus not only a marker for the oncogenicity of ASV, but can also be used in its partially purified form for successful anti-tumour immunisation (H. Bauer and M. Hayami, personal communication). Here we have demonstrated that ALV infection, even in a cell already possessing a transformed phenotype, does not lead to the expression of TSSA.

The sole important genetic difference in the genomes of ASV and ALV is the sarc-gene of ASV, which is not part of the ALV genome and seems to be responsible for fibroblast transformation¹⁵⁻¹⁷. We are aware of no experimental evidence contradicting the notion that expression of the sarc-gene inevitably leads to the expression of TSSA in the plasma membrane of fibroblasts. It can, but does not necessarily mean that TSSA is coded for by the sarc-gene and thus represents a primary transforming protein. It is also possible that the primary transforming protein coded for by the sarc-gene induces TSSA synthesis by cellular genes. To distinguish between the

latter two possibilities, *in vitro* protein synthesis experiments have been initiated to attempt the transcription of the protein portion of TSSA from ASV-RNA *in vitro*.

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Immunological identification of two adenovirus 2-induced early proteins possibly involved in cell transformation

HUMAN adenovirus 2 (Ad2), a DNA tumour virus that replicates in the nucleus of permissive human cells^{1,2}, transforms non-permissive or semipermissive cultured rodent cells, but does not induce tumours in newborn hamsters (oncogenic group C). The constant functioning of protein product(s) from Ad2 'transforming gene(s)' is probably responsible for the transformed phenotype, although this has not been proved. Identification and functional characterisation of 'transforming protein(s)' are important for the understanding of cell transformation and growth control. We describe here immunoprecipitation studies made with antisera against presumptive Ad2 'transforming protein(s)', that identify two candidate transforming proteins of 53,000 (53k) and 15kdaltons.

Ad2-transformed cells retain viral DNA and synthesise viral RNA sequences that are a subset of those produced during 'early' stages of productive infection, before initiation of viral DNA replication³. All Ad2-transformed cells examined contain the left 14% of the Ad2 genome, that is map positions (MP) 0-0.14 (ref. 4). These cells express mRNA from Ad2 DNA about MP 0.02 to 0.10 assuming a continuous transcript, that are also expressed

early⁵. Some Ad2-transformed cells contain and express other sections of the viral genome, but none contain the entire genome^{4,5}. Cultured rat cells are transformed by transfection with G fragment (MP 0-0.075) generated by cleavage of Ad2 or Ad5 (oncogenic group C) DNA with restriction endonuclease *Hind*III, and digestion of more than 1% of DNA by exonuclease III + *S*₁ nuclease results in loss of transforming activity⁶. These studies indicate that information for cell transformation lies somewhere between Ad2 MP 0.01 and 0.075, and is expressed early after infection.

To identify Ad2 transforming protein(s) we prepared antisera against viral proteins expressed in Ad2-transformed rat cells by injecting sonicated F17 cells into rats. Cell line F17 (P. H. Gallimore) contains only the left 14% of the Ad2 genome⁴, and thus viral proteins should include and be mainly transforming proteins. We also used antisera from hamsters immunised with extracts of tumours induced in hamsters by Ad2-SV40 and Ad1-SV40 (Ad1 is in oncogenic group C) hybrid viruses (antisera were obtained from Dr R. Gilden⁷). The Ad2 DNA sequences present in the Ad1- and Ad2-SV40-induced tumours have not been determined. IgGs from F17 and hamster antisera were used to immunoprecipitate ³⁵S-labelled polypeptides produced in human KB cells early after productive infection. Polypeptides were identified by polyacrylamide slab gel electrophoresis and

autoradiography. Injection of Ad2-transformed rat or hamster cell extracts into rats or hamsters, respectively, should minimise induction of antibodies to rat or hamster proteins, and therefore decrease the danger of immunoprecipitation of human proteins from Ad2-infected KB cells by possible cross-reacting antibodies to rat or hamster proteins. Accordingly, proteins specifically immunoprecipitated by F17, Ad1-, or Ad2-SV40 IgG from Ad2-infected KB cell extracts should be virus specific.

In studies of Ad2 early proteins cells are treated with arabinosyl cytosine (ara C) 4h after infection and labelled in the presence of ara C; this prevents viral DNA replication and consequently the onset of late stages of infection, so that only early viral polypeptides are labelled. Two polypeptides (75k DNA binding protein, 21k) are in infected cell extracts (Fig. 1b), that are absent from uninfected cells (Fig. 1a). Treatment of cells with cycloheximide before labelling enhances viral protein synthesis relative to host⁸. In this gel 5 Ad2 induced early proteins are visible after cycloheximide pretreatment (Fig. 1d): 75k, 21k, 19k, 15k, and 11k (a 11.5k polypeptide forms part of the 11k band). Using longer gels (27cm) with higher resolution we have observed as many as 13 Ad2-induced early polypeptides, but do not know whether each is a product of a unique viral gene.

Immunoprecipitation studies using F17 IgG are shown in Fig. 1, e-l. Cells were not pretreated with cycloheximide, and were

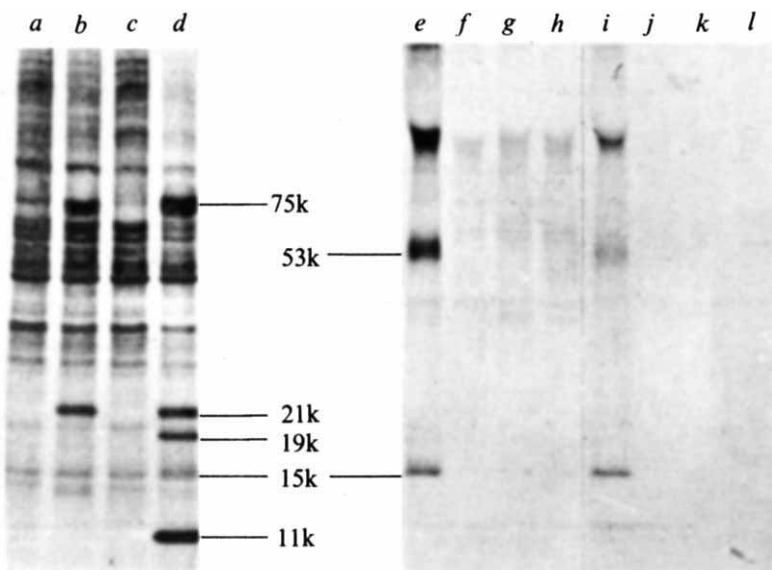
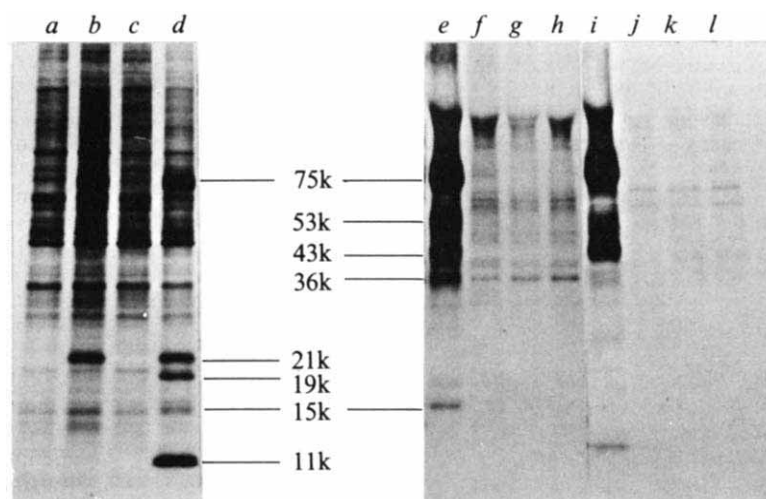


Fig. 1 Autoradiogram of sodium lauryl sulphate-polyacrylamide slab gel showing electrophoretic separation of ³⁵S-polypeptides induced in human KB cells by Ad2 early after infection, and immunoprecipitated by rat antiserum against extracts of Ad2-transformed F17 rat cells. KB cells suspended in Eagle's MEM at 6×10^6 cells per ml were infected with 100 plaque-forming units per cell of Ad2^{9,10}. A second culture of KB cells was treated in a similar manner in this and subsequent steps, but without addition of virus, to serve as a mock-infected control. After 1h adsorption cells were diluted to 3×10^5 cells per ml with MEM containing 5% horse serum and 10 mM HEPES, pH 7.2. Four hours after infection arabinosyl cytosine (ara C) was added to $20 \mu\text{g ml}^{-1}$. At 6h after infection cells were resuspended at 9×10^5 cells per ml in methionine-free MEM containing 5% horse serum, 10 mM HEPES, pH 7.2, and ara C ($20 \mu\text{g ml}^{-1}$), and labelled for 6h with ³⁵S-methionine ($25 \mu\text{Ci ml}^{-1}$ 273 Ci mmol⁻¹). After labelling, cells were washed twice by centrifugation with ice-cold phosphate-buffered saline (PBS), suspended at 18×10^6 cells per ml in reticulocyte standard buffer, allowed to swell for 15 min, and disrupted by 30 strokes of a tight-fitting Dounce homogeniser. Nuclei were collected by centrifugation and the perinuclear membrane was removed by the 'double-detergent' washing procedure¹⁴ using Tween 40 and sodium deoxycholate. Nuclei were suspended in radioimmune precipitation (RIPA) buffer¹⁵, sonicated for 3 min, incubated at 20°C for 150min, and clarified by centrifugation (4°C) at 31,000g for 20 min. RIPA buffer is 10 mM sodium phosphate, pH 7.2, 150 mM NaCl, 1% Triton X-100, 1% sodium deoxycholate and 0.1% sodium lauryl sulphate. The postnuclear cytoplasm was mixed with 0.1 volume of $10 \times$ RIPA buffer, incubated at 20°C for 15 min, and clarified as described for nucleoplasm. F17 cells for immunisation were grown in Ca²⁺-free MEM supplemented with 10% foetal calf serum. Before each injection, cells were collected by scraping with a rubber policeman, washed three times in PBS, and sonicated for 3 min. A sonicated 10% cell suspension in PBS was emulsified with 1 volume of Freund's complete adjuvant and 0.5 ml of emulsion was injected intraperitoneally into each rat. Eight injections were performed at weekly intervals and rats were exsanguinated 9d after the last injection. Immune sera from four Sprague-Dawley and three Wistar rats were pooled. IgG was isolated from the F17 serum pool (F17 IgG) or from a pool of normal rat sera (non-immune IgG) by standard methods as previously described^{9,10}. Samples from the infected or mock infected cytoplasmic (9×10^6 c.p.m.) or nuclear (3×10^6 c.p.m.) extracts were mixed with 30 μg of F17 IgG or non-immune rat IgG in 150- μl reaction mixtures containing RIPA buffer. Reaction mixtures were incubated in 0.4-ml plastic disposable centrifuge tubes (Beckman) for 18h at 4°C. Goat serum anti-rat IgG (28 μl) was added and the incubation continued for 2h at 37°C. The contents of the tubes were layered on 180 μl sucrose (10% sucrose in RIPA buffer) and centrifuged for 1.5 min at 10,000g in a Beckman 152 microfuge. The supernatants were removed by suction and the precipitates were washed three times by vigorous mixing with 150 μl of RIPA buffer, then resuspended in 20 μl of sample buffer⁸ without 2-mercaptoethanol. Radioactivity in an aliquot of each sample was determined. An average of 0.26% of input counts was in control precipitates (infected cytoplasmic extracts against non-immune IgG, or mock infected cytoplasmic extracts against both non-immune and F17 IgG) whereas 0.69% of input counts were in precipitate from infected cytoplasmic extract against F17 IgG. The precipitates of the corresponding controls of the nuclear extracts contained an average of 0.64% of input counts, whereas the precipitate from infected nuclear extract against F17 contained 1.66% of input counts. Equal volumes (7 μl) from each precipitate suspension were boiled for 4 min and analysed by sodium lauryl sulphate polyacrylamide gel electrophoresis in 9-10-cm gradient slab gels (8-21%) as previously described⁸ except that slabs were 0.75 mm thick. Extracts of mock-infected and Ad2-infected cells were also included to serve as markers. These were obtained as described above except that cells were labelled with ³⁵S-methionine ($25 \mu\text{Ci ml}^{-1}$) for 1h only (7.5-8.5h after infection). Two other cultures, infected and mock infected, were prepared by the cycloheximide enhancement method⁸. Briefly, cycloheximide ($25 \mu\text{g ml}^{-1}$) was added 1h and ara C ($20 \mu\text{g ml}^{-1}$) at 4h after infection. At 7h after infection cells were washed twice with warm medium containing ara C ($20 \mu\text{g ml}^{-1}$), and labelled for 1h with ³⁵S-methionine as described above. The cells were collected, washed with ice-cold PBS, precipitated with 10% trichloroacetic acid, solubilised in sample buffer, and electrophoresed on polyacrylamide gels as before⁸. After electrophoresis, gels were dehydrated in a solution of dimethyl sulfoxide, soaked in a solution of 2,5-diphenyloxazole in dimethyl sulfoxide¹⁶, dried, exposed to film at -70°C, and autoradiographs were developed. a, Uninfected cells, ara C-treated; b, infected cells, ara C-treated; c, uninfected cells, CH- and ara C-treated; d, infected cells, CH- and ara C-treated; e, infected cytoplasmic extract against F17 IgG; f, infected cytoplasmic extract against non-immune rat IgG; g, uninfected cytoplasmic extract against F17 IgG; h, uninfected cytoplasmic extract against non-immune rat IgG; i, infected nuclear extract against F17 IgG; j, infected nuclear extract against non-immune rat IgG; k, uninfected nuclear extract against F17 IgG; l, uninfected nuclear extract against non-immune rat IgG.

fractionated into cytoplasm (*e-h*) and nucleoplasm (*i-l*). F17 IgG specifically precipitated 51–55k (average 53k) and 15k polypeptides from both cytoplasmic (Fig. 1*e*) and nuclear (Fig. 1*i*) extracts of infected cells. IgG from unimmunised rats (non-immune rat IgG) did not precipitate these polypeptides from infected cell cytoplasmic (Fig. 1*f*) or nuclear (Fig. 1*j*) extracts. Neither F17 nor non-immune rat IgG precipitated 53k and 15k polypeptides from uninfected cell extracts (Fig. 1*g* and *k*, and *h* and *l*, respectively). Several minor host bands were precipitated nonspecifically by all IgG samples. The swelling at the top of the lanes of Fig. 1 representing immunoprecipitated samples is caused

polypeptides⁸. We have observed, however, a 53k polypeptide in infected cells by labelling in hypertonic medium (unpublished results), which greatly reduces the synthesis of host proteins relative to viral proteins. Thus both 53k and 15k polypeptides can be detected, in special labelling conditions, that could correspond to the immunoprecipitated polypeptides. We have also observed 53k and 15k in various protein labelling and immunoprecipitation experiments: (1) with and without pretreatment of cells with cycloheximide; (2) with cells labelled between 5 and 8h, 7 and 7.5h, 8 and 11h (but not 2 and 5h), 6 and 10h followed by a 12-h chase, and both with and without 2 mM phenyl-

Fig. 2 Autoradiogram of polyacrylamide slab gel, showing electrophoretic separation of polypeptides precipitated by Ad1-SV40 IgG from cytoplasmic and nuclear extracts of Ad2 early infected KB cells. The same markers and extracts described in the legend to Fig. 1 were used, and precipitated in the same manner, except that 20 μ g of Ad1-SV40 IgG or non-immune hamster IgG was used. Goat serum anti-hamster IgG (10 μ l) was added after 18h of incubation at 4°C. Fraction of input counts recovered in precipitates: cytoplasmic controls, average 0.23%; infected cytoplasmic extract against Ad1-SV40 IgG, 1.58%; nuclear controls, average 0.61%; infected nuclear extract against Ad1-SV40 IgG, 3.99%. Placement of the lanes is identical to that described in Fig. 1 except that Ad1-SV40 IgG replaced F17 IgG and non-immune hamster IgG replaced non-immune rat IgG.



by IgG (150k). In this experiment samples were not treated with 2-mercaptoethanol to reduce disulphide bonds as this dissociates IgG into 50k and 25k subunits that distort the lower gel portions. Mercaptoethanol treatment did not affect the 53k and 15k bands, nor reveal any additional virus-specific polypeptide bands. The protein band immediately below the IgG in Fig. 1*e* and *i* was not present when mercaptoethanol was used, and therefore probably is an artefact of antigen-antibody dissociation or electrophoresis, and not a high molecular weight virus induced polypeptide.

As Fig. 2 shows, Ad1-SV40 IgG also specifically immunoprecipitated 53k and 15k polypeptides from the cytoplasm of infected cells (Fig. 2*e*), and 53k but not 15k from the nucleoplasm (Fig. 2*i*). The Ad1-SV40 titre to 15k seems to be lower than that of F17, since it precipitated less 15k from the cytoplasm, and apparently none from the nucleus. Neither 53k nor 15k were precipitated from uninfected cell extracts by Ad1-SV40 IgG (Fig. 2*g* and *k*), or from infected (Fig. 2*f* and *j*) or uninfected (Fig. 2*h* and *l*) cell extracts by non-immune hamster IgG. Ad1-SV40 IgG also precipitated large amounts of 75k, as expected from our earlier studies with Ad1- and Ad2-SV40 antisera proving that 75k is expressed in the Ad1- and Ad2-SV40-induced hamster tumours^{9,10}. Also precipitated were diffuse bands of 43k and 36k polypeptides (Fig. 2*e* and *i*), which are degradation products of 75k (ref. 11 and unpublished data). Ad2-SV40 IgG gave identical results (data not shown). Saborio and Öberg¹² reported that Ad2-SV40 IgG precipitated 72k, 67k, 60k, and 45k polypeptides from extracts of labelled Ad2-infected HeLa cells. How these correspond to the polypeptides we have observed is not known. It is possible that they did not detect our 15k polypeptide because of the low titre of Ad2-SV40 IgG against this polypeptide.

Unequivocal identification of the 53k and 15k polypeptides as virus-specific is difficult by radioimmune precipitation and gel electrophoresis, because of possible cross-reacting antibodies, nonspecific precipitation and proteolytic breakdown of immunologically reactive viral proteins. At 15k polypeptide is visible in extracts without immunoprecipitation, especially after cycloheximide pretreatment, but a 53k polypeptide usually cannot be resolved from the large background of host

methylsulphonylfluoride (protease inhibitor); (3) with two independent pools of F17 antisera; (4) with and without absorption of F17 antiserum with extracts of uninfected KB cells. In these various experiments 53k was observed as a fairly diffuse band as in Figs 1 and 2. Some variability was observed in the polypeptides in the 15k region of gels. Occasionally a polypeptide of about 17k was observed in cytoplasmic extracts (Fig. 1*e*), and one of 11k in nuclear extracts. The 11k polypeptide apparently induced by Ad2 and localised in the cell nucleus (unpublished), was also precipitated by non-immune IgG, probably nonspecifically. Also the 15k polypeptide band sometimes appeared as a doublet and even as a triplet. The significance of this variability is unclear: the 'extra' bands may represent some type of modification of 15k. It is also possible that F17 and the Ad SV40 antisera contain low titres of antibodies against viral polypeptides of 15-17k in addition to 15k.

Our major finding is that three antisera (F17, Ad1-SV40 and Ad2-SV40) expected to contain antibodies against Ad2 transforming protein(s) specifically precipitate 53k and 15k polypeptides. The synthesis of both polypeptides in Ad2-transformed rat cells, Ad-SV40 hamster tumour cells, and Ad2-infected human cells, suggests (but does not prove) that they are virus coded. Since F17 contains only the left 14% of the Ad2 genome⁴, which includes transforming genes, these polypeptides are strong candidates for transforming proteins. It is important to note that F17 cells apparently express Ad2 mRNA sequences in addition to those of presumptive transforming genes; that is Ad2 transforming genes map somewhere within MP 0.01 and 0.075 (ref. 6), whereas F17 may express roughly MP 0.03-0.10 as mRNA⁵. It is therefore possible that either 53k or 15k (or both) is not a transforming protein. The only evidence available on this point is from Lewis *et al.*¹³, who have translated *in vitro* Ad2 mRNA, hybridisation-purified from F17 cells, into a diffuse 44-50k and a prominent 15k polypeptide. The 15k polypeptide was produced by translation of mRNA selected by hybridisation to either *Hind*III-G (MP 0-0.075) or *Hind*III-C (MP 0.075-0.17), but not *Hpa*I-E (MP 0-0.041). As *Hind*III-G can transform cells this argues that the 15k polypeptide may not be a transforming protein, unless there is more than one 15k, or unless the 3'-

terminal sequences (complementary to *Hind*III-C) in the mRNA for the 15k polypeptide are superfluous. The 44–50k polypeptide mapped to the left of 15k and thus its gene is closer to the transforming region. Of course it is not known whether our 53k and 15k correspond to the 44–50k and 15k polypeptides of Lewis *et al.*, but if so, then 53k may be a better candidate than 15k as a transforming protein. Positive identification of Ad2-transforming protein(s) will require transformation-defective mutants, as well as antisera against transformed cells that express only transforming gene(s).

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Growth regulation of cells grown in suspension culture

VARIOUS aspects of a cell's growth cycle can be manipulated in tissue culture. For example, the initiation of DNA synthesis and, in some cases, culture growth, can be enhanced by serum¹ and platelet² components, polypeptides purified from growth-conditioned medium³ and urine⁴, insulin⁵, corticosteroids⁶, prostaglandins⁷, cyclic nucleotides (see, for example, ref. 8) and proteolytic enzymes⁹. In addition, at least three purified proteins can initiate DNA synthesis in fibroblast-like cells—nerve growth factor¹⁰, epidermal growth factor¹¹ and fibroblast growth factor^{12,13}. Disturbance of the culture medium directly over the cultured cells also induces DNA synthesis¹⁴. Fibroblast-like cells have been used in most of these studies, and they grow tightly associated with the culture dish and are strongly anchorage dependent for division^{15,16}. Given the strong anchorage dependence of the test cells, two alternatives could account for the induction of DNA synthesis and growth by the diversity of conditions and "factors" outlined above. The first would ascribe the effects of these compounds to interactions on the cell surface with a suitable receptor. These are hormone-like interactions, thought to be exemplified by compounds such as insulin and purified

growth factors¹. The second alternative represents interactions which function directly through alterations of cell anchorage. For example, the growth stimulating effects of the proteases are presumably affected by modification of the cell-substratum (anchorage) interaction¹⁷. To distinguish between these two alternatives, it should be sufficient to assay the effects of serum and a purified growth factor on the initiation of DNA synthesis in an anchorage-dependent cell line, and on a variant which grows in suspension culture. If serum and the growth factor were equally effective in both cell lines, then a disturbance in anchorage could not account for the mitogenic activity, ruling out the second alternative. The first, hormone-like, mechanism, however, would be excluded if the growth factor was unable to induce DNA synthesis in the cells grown in suspension. The following experiments support this latter possibility by showing that fibroblast growth factor^{12,13} does not affect the growth of myoblast cells grown in suspension culture.

The cells examined were a rat myoblast line (L6) derived from embryonic skeletal muscle¹⁸ and a subline (M3A) derived from it which grows in suspension culture¹⁹. Although M3A does not fuse to form myotubes, its growth characteristics and regulation of some muscle enzymes are the same as the attached parent cell line¹⁹. When tested in several concentrations of serum, the serum dependence for growth of M3A and L6 cultures is similar, with the M3A cells responding slightly less well to low serum concentrations (Fig. 1). Finally, 3T3A4, an anchorage-dependent line whose response to various growth factors has been well defined²⁰, was used as a control to test the efficiency of the purified growth factor.

A growth promoting factor for fibroblast cells, initially found in pituitary extracts¹² and subsequently purified¹³, was used because it seems to initiate DNA synthesis in a wide variety of cell types¹³. This factor, termed fibroblast growth factor (FGF), was purified²¹ and used in the following studies. The assay for the initiation of DNA synthesis was done on sparse quiescent cells²⁰. Table 1 shows that purified FGF stimulated the incorporation of ³H-thymidine by resting 3T3 cells as described before²⁰, demonstrating that the FGF preparation was functional on the fibroblasts.

When attached L6 myoblasts were tested for stimulation of DNA synthesis by FGF in conditions identical to those described for 3T3, there was an increased incorporation of ³H-thymidine into trichloroacetic acid (TCA)-precipitable material (Table 1). The greatest stimulation was with FGF

Table 1 Effect of FGF on fibroblasts, anchorage-dependent myoblasts and suspension grown myoblasts

Additions	Cell lines and relative increase in thymidine incorporation		
	3T3A4	L6	M3A
None	1	1	1
10% Serum	17.1	7.1	5.8
FGF	4.5	1.4	1.1
Insulin	—	2.0	1.0
Hydrocortisone	1.0	1.1	0.9
Hydrocortisone + insulin	3.3	2.1	1.1
FGF + insulin + hydrocortisone	6.6	7.0	1.2

Cells were plated in 10% foetal calf serum, and after 2 d the serum concentration was reduced to 0.5%. 48 h later the indicated reagents were added to the growth-inhibited cells (density between 5×10^4 and 2×10^5 cells per 60-mm dish). 18 h later the incorporation of ³H-thymidine into TCA-precipitable material was assayed as described²⁰. The concentrations of the reagents (ng l^{-1}) were: FGF, 100; insulin, 1,000; hydrocortisone, 1,000. Data are presented as the increase, relative to control cultures in 0.5% serum, of the ³H-thymidine incorporation. Background incorporation in control cultures was 5,100 c.p.m. for 3T3A4, 6,800 c.p.m. for L6 and 7,900 c.p.m. for M3A. The data are the means of triplicate determinations, with an average error between samples of less than 10%. The experiment was repeated three times with similar results.

in combination with insulin and hydrocortisone. But when the suspension-grown cell line, M3A, was assayed in the same conditions there was little or no stimulation of ^3H -thymidine incorporation with the defined reagents (Table 1), although the stimulation achieved by the addition of whole serum was similar to that found with L6. Thus the combination of FGF, insulin and hydrocortisone, compounds which act synergistically with FGF to stimulate DNA synthesis 3T3 cells²⁰, stimulate anchorage-dependent L6 cells but not their suspension-grown variant. Since both L6 and its anchorage-independent derivative M3A are responsive to serum stimulation to approximately the same extent (Fig. 1 and Table 1), the possibility that FGF and its synergists can replace the serum requirement of these cells is ruled out. In addition, if M3A had lost its hypothetical surface receptors for FGF, then FGF has no rate limiting physiological role in the growth regulation of these cells by serum.

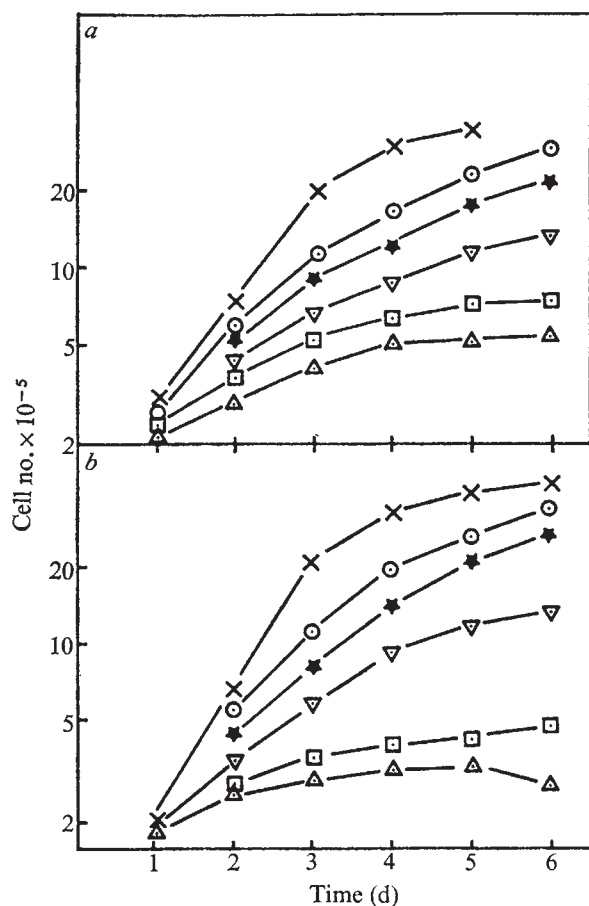


Fig. 1 Serum dependence for culture growth of L6 and M3A cells. Cells were plated on day 0 in 60-mm tissue culture (L6) or Petri dishes (M3A) in 5 ml of medium containing the indicated concentration of foetal calf serum. The cell number was then followed as a function of time. *a*, L6; *b*, M3A; Δ , 0.2% serum; $\square$, 0.5% serum; ∇ , 1% serum; $\star$, 2% serum; $\circ$, 5% serum; $\times$, 10% serum.

If it is generally true that cells grown in suspension culture are not sensitive to the defined "factors" which stimulate the growth of attached, anchorage-dependent cells, then some caution should be exercised in interpreting results based exclusively on attached cells. Of the conditions that tend to stimulate growth of attached fibroblast-like cells, there seems to be one underlying factor—the disturbance of the limiting cell membrane. For example, insulin stimulates growth^{3,20} and is weakly proteolytic²²; the corticosteroids are lipophilic and interact nonspecifically with membranes. Nerve growth factor stimulates the growth of fibroblast-like cells, presumably through its proteolytic subunit¹⁰, as do

various proteolytic enzymes^{9,17,23}. Since overt proteolytic treatment of anchorage-dependent cells inhibits division by rendering the cells into suspension, it follows that the extent of proteolysis that initiates cell division is quantitatively less. In addition, disturbance of the medium above cells triggers DNA synthesis¹⁴, perhaps perturbing the cell-substratum interactions by exerting a weak shear force on the cells. Various inorganic ions are nonspecifically mitogenic²⁴, perhaps for the same reason. As the degree of cell anchorage is critical in the initiation of cell division (see, for example ref. 17), it is possible that any reagent which disturbs this cell surface interaction in attached cells may trigger one or more rounds of DNA synthesis and cell division. Such a reagent need not be proteolytic. For example, several purified proteins lacking proteolytic activity induce neurite retraction in C1300 neuroblastoma cells, presumably by weakening the cell-substratum interaction required for neurite extension²⁵.

In summary, if purified (serum) growth factors are functioning in a hormone-like manner by interacting with specific cell surface receptors, then one would predict that anchorage-dependent and independent lines should respond similarly if their serum response is the same. As this hypothesis is contradicted by the data reported here, it is possible that "growth factor" responses of attached cells are not mediated through specific surface receptors, but through less specific perturbations of the cell-substratum interaction. For anchorage-dependent cell lines, anchorage is necessary but not sufficient to regulate cell division. By using anchorage-independent cells for the characterisation of growth promoting activities, it is possible to eliminate one experimental variable.

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Effect of 3-acetylpyridine on tissue differentiation of the embryonic chick limb

3-ACETYLPIRIDINE (3-AP) and 6-aminonicotinamide have a teratogenic effect on the development of the chick limb but have no effect of coadministered with nicotinamide¹. This

has led Caplan to the hypothesis that nicotinamide has a crucial role in the spatial organisation of muscle and cartilage in the developing limb, high levels of nicotinamide specifying muscle, and low levels cartilage; with even the possibility that a metabolic gradient related to the blood supply could provide the positional information specifying the pattern of muscle and cartilage²⁻⁵. The evidence for this hypothesis is based both on *in vivo* and *in vitro* studies, the latter claiming to show that 3-AP enhances chondrogenesis and inhibits myogenesis in cultured limb mesenchyme cells. As regards the *in vivo* effects, it is suggested that the gross muscle hypoplasia observed might be accounted for in terms of 3-AP switching the differentiation of mesenchyme cells from a myogenic fate to a chondrogenic one. This seemed to us to be an unlikely explanation, since the chondrogenic and myogenic tissues are already physically separated at stages when 3-AP is still known to have an effect (as late as 13 d of incubation). It seemed much more likely that 3-AP was affecting muscle growth rather than its differentiation. We therefore made a histological study of the effect of 3-AP, and found that it was a potent destroyer of the peripheral nerves, and that there is no evidence to support the view that it affects mesenchyme differentiation.

Eighty-four White Leghorn embryos at 3 d of incubation were windowed and sealed with Sellotape. At 6 d of incubation, 2 mg of 3-AP (Sigma) in 100 μ l of distilled water were dropped on to the embryo membranes of 56 of these, the remainder serving as controls. The eggs were resealed and incubated for periods from 6 h to 5 d when the embryos were removed and the right wings and legs fixed in Karnovsky's solution and embedded in Araldite. Transverse sections (1 μ m) were taken from a region of the leg 1.5 mm proximally from the distal end of the tibia, where the thin appearance of treated limbs is most marked, and from the wing mid-way along the radius and ulna, limbs being measured and marked in Araldite before cutting. The sections were stained with Toluidine blue, and areas measured from camera lucida drawings with a Stanley Planimeter. By 3 d after treatment, the usual gross morphological appearance of 3-AP treatment (described as 'muscle hypoplasia' and evident particularly as thinness of the legs) was discernible, and by 5 d it was marked, with embryos being

reduced in wet weight by up to 30% and their legs, although of approximately normal length, reduced by up to 50% in thickness. As is usual in both teratogenic and genetic defects, the legs were affected much more markedly than the wings, and there was considerable variation in expression of the defects, ranging from almost normal appearance to death.

At 6 d of incubation, when treatment began, the cartilage of both wing and leg is well differentiated but ossification has not yet begun. The dorsal and ventral muscle masses are clearly visible, and the splitting division into individual muscles is just beginning to be evident, but the tendons have not yet begun to develop. The physical separation of chondrogenic from myogenic tissues should be stressed, the cartilage being surrounded by a well defined perichondrium. The nerves at this stage extend as far as the wrist and ankle. Sections were taken from five legs which had been left 5 d after treatment, together with five equivalent controls (Fig. 1). Notable is the absence of tendons, and the reduction in the area of the cartilage of the treated animal (49% reduction in the average area of the tibia). It is these factors, together with the reduction in the loose mesenchyme which give the limb its characteristic thin appearance at this stage; there is in fact virtually no muscle normally present in this region so the usual description of 'muscle hypoplasia' is inappropriate. Further, nerves could not be found in the treated legs, although they were well developed in the controls.

This last result has been noted previously by Tanaka *et al.*⁶, although they remained uncertain as to its exact importance. The effect is apparent on peripheral rather than on spinal nerves, although the neurotoxic effect of 3-AP on adult brains has been noted by several authors⁷.

Since 3-AP is described as specifically affecting differentiation of the muscles, sections were taken from the muscle-rich region of the wing described above. Sixteen treated wings at various stages were examined, together with 12 controls. The muscle up to 2-3 d after treatment was substantially normal, though somewhat smaller than the controls; and the muscle blocks began to split up, again as normal, giving rise to individual muscles. After 3 d the muscles began to degenerate. There was no significant

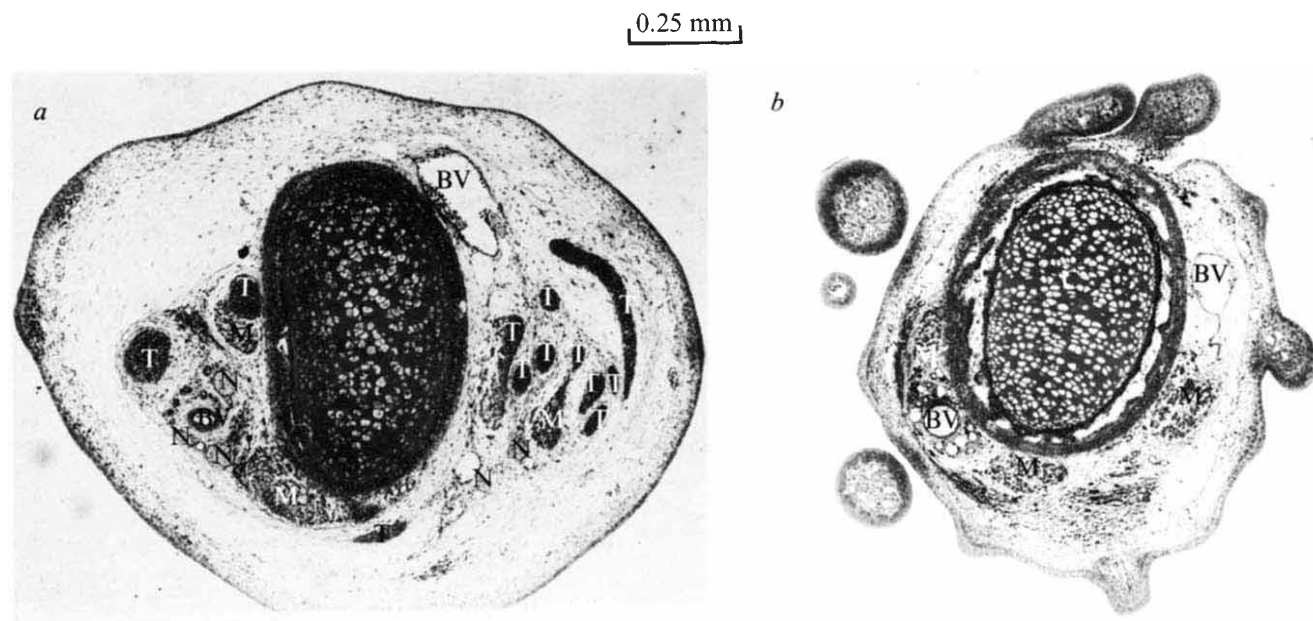


Fig. 1 C, Cartilage; M, muscle; T, tendon; N, nerve; BV, blood vessel. *a*, Transverse section of leg of 11-d-old control animal. The cartilage element is the tibia. *b*, Transverse section of leg of animal treated with 3-AP at 6 d, 5 d after treatment. Tendons and nerves are absent and the cartilage is smaller in area.

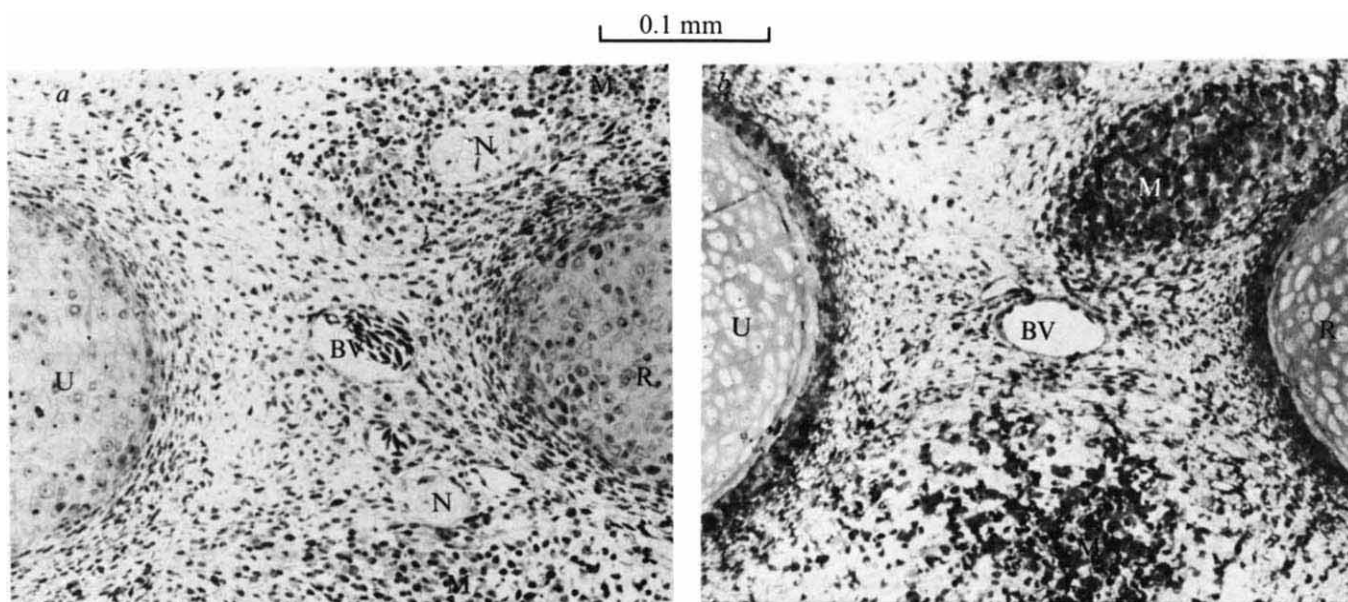


Fig. 2 R, Radius; U, ulna; N, nerve; M, muscle; BV, blood vessel. *a*, Enlargement of central region of transverse section of normal 6-d-old chick wing (that is, at time of treatment). Nerves are clearly present. *b*, Same region of limb treated with 3-AP, 24 h after treatment (that is, at 7 d of incubation). The nerves present at 6 d have now been destroyed, but the muscle blocks are not obviously affected.

reduction in the area of the cartilage; the most striking effect was on the nerves. In embryos examined 6–12 h after treatment, some nerves were already absent. Examination at 24 h or later showed all limbs except one to be completely devoid of nerves (Fig. 2).

These results show that 3-AP has a deleterious effect on the growth of all the tissues in the developing limb, that on nerves being the most marked. Since it is well known that denervation results in muscle degeneration^{8,9}, the long term effect of 3-AP on muscles is most probably mediated by its effect of destroying peripheral nerves within about 24 h of treatment. The absence of tendons may also have an adverse effect on muscle development. 3-AP can also reduce the radial growth of cartilage viewed in cross section, although the length of the cartilaginous elements is not affected, perhaps because of interference with cell division, leaving matrix secretion unaffected. Conversely, our preliminary results suggest that 6-AN does affect matrix secretion. The greater effect on the leg when compared with the wing, can be related to the comparatively small amount of radial growth in the radius and ulna during the experimental period compared with the tibia (twofold as opposed to ninefold for the latter).

The effect of 3-AP on the tissues of the limb bud as revealed by histology shows clearly that it does not act specifically by repressing myogenesis and enhancing chondrogenesis in the manner proposed by Caplan, but rather indicates a deleterious effect on various cell types including cartilage, mesenchyme, muscle and tendon, but which is particularly marked in the case of peripheral nerves, these being almost totally destroyed after only 24 h treatment, and whose absence must lead to serious defects in the later development of the muscles. The biochemical mechanisms involved in the specification of muscle and cartilage remain quite unknown.

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Development of the locust ocellus

WORKERS interested in retinal development have been attracted by the iterative unit structure and prolonged development time of the compound eye of hemimetabolic insects^{1–4}. By comparison, the ocellus of the adult locust (*Schistocerca gregaria*) is a simple retina composed of only 1,000 receptive elements with a very loose cartridge-like division of receptors and underlying synaptic plexus. The plexus is composed of receptor terminals, the arborisations of second-order cells and glial elements. Most of the second-order contributions to this structure are composed of the repeatedly branched endings of the so-called giant cells, on to which the retina is highly convergent. The structure is so simple that here I report the possibility of examining developmental mechanisms at the single-cell level.

In eggs maintained at $28^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$ the ocelli first become visible through the embryonic cuticle as small white patches about 178 h before emergence (mean total development time 380 h). Sections cut at this stage show the ocelli as cone-shaped aggregations of 'tailed' epidermal cells. No distinct growth cones are present and few neurotubules are found in the cytoplasm. By 165 h before emergence the epidermal cells have extended 60–70 axonal processes $225\text{ }\mu\text{m}$ long, deep into the brain. This gives the embryonic retinula axons a growth rate of about $17\text{ }\mu\text{m h}^{-1}$, somewhat slower than the $25\text{ }\mu\text{m h}^{-1}$ recorded for *Lucilia* compound eye retinula cells¹. The axons are rich in neurotubules and the cytoplasm contains an abundance of ribosomes and granules.

On entering the embryonic neuropile the axons establish conical growth cones rich in filopods. During growth to this point growth cones are typically spear shaped. Within the brain the filopods of the retinula cells make electron dense contact with certain neuropilar processes. At this

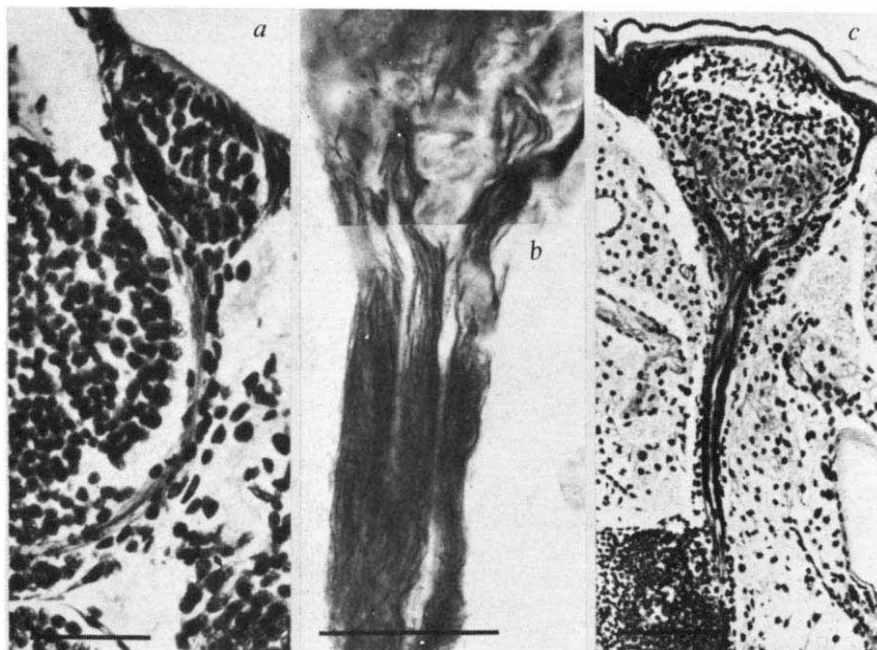


Fig. 1 *a*, A 10- μ m wax section, stained by the Power-Bodian technique⁷, of the lateral ocellus of a newly emerged hopper. The ocellar nerve containing about 70 retinula cell axons skirts the protocerebral lobe and enters the protocerebrum near the midline. Most such axons terminate in the dorsal pars intermedia. Many of these axons are derived from rhabdomeless cells and thus cannot be functional at this stage. The bar represents 50 μ m. *b*, The retinal axons of the newly emerged hopper appear tightly grouped around the second-order axons, only three of which lie within this section. The bar represents 25 μ m. *c*, The lateral ocellus of a second instar locust during the intermolt period. Note the vast expansion in the number of cells in the retina; retinula cell axons are absent from the length of the ocellar nerve. Branches pass from the second-order cells into the descending retinal bundles. The darkly stained group of cells (top left) are thought to represent the next group of retinula cells to be recruited from the surrounding epiderm. The bar represents 100 μ m.

stage no second-order processes can be detected in the descending bundle of retinula cell axons.

Sections taken shortly before emergence (380 h) show the presence of five large second-order cell profiles in the

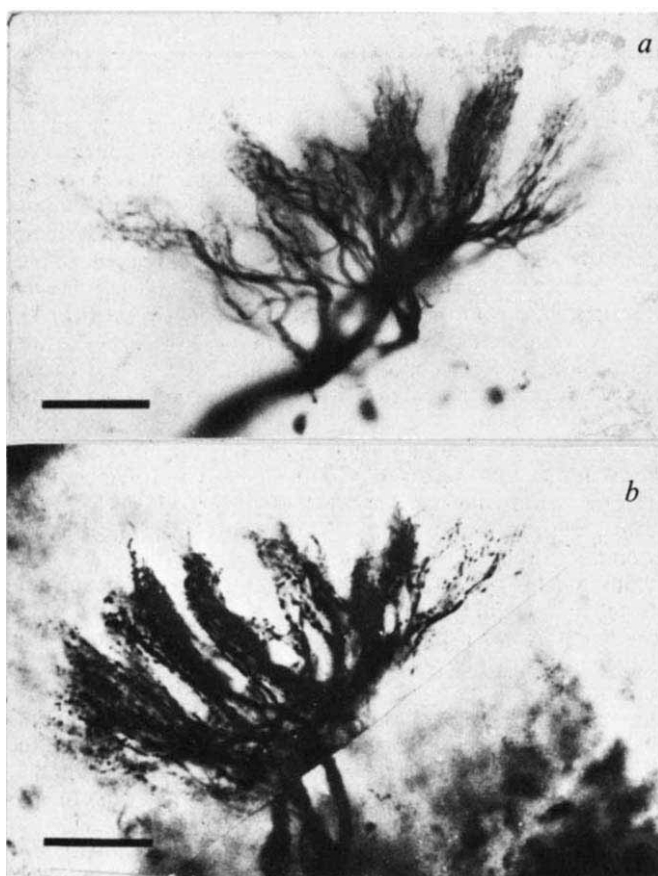


Fig. 2 *a*, Cobalt stain of the two latero-median axons viewed as a whole mount of the adult lateral ocellus. *b*, Whole mount of an adult lateral ocellus which contains a third latero-median axon. In spite of the presence of an extra nerve there is no commensurate increase in the total number of collaterals generated. The bars represent 100 μ m.

ocular end of the lateral ocellar nerve. The second-order profiles are readily distinguished from the receptor terminals because of their white cytoplasm. Two hours after emergence the retina cell axons still extend within the brain but only to the top of the protocerebral lobe (Fig. 1*a, b*). This positional change is not due to any change in length of the retinula cells but rather to a sustained growth of the second-order axons and an associated increase in the length of the ocellar nerve. Synapse-like structures are present in the ocellar nerve 6 h before emergence but usually in association with dense-cored vesicles.

After emergence into the light, synaptic structures proliferate rapidly. The lateral ocellar second-order axons extend in the course of the first two instars until all the retinula cell axons lie within the proximal half of the ocellar nerve (Fig. 1*c*). New retinula cells are recruited from the surrounding epiderm at each moult, and their axons are incorporated into the synaptic plexus.

Examination of silver-stained, semi-thin and thin sections shows increase in the complexity of the second-order giant cell arborisations with each moult, retinula cell ingrowth apparently preceding the addition of branches to the giant cells. The presence of two axons connecting the median ocellus with the lateral ocellus^{5,6} makes possible the staining of these two cells' arborisations by the diffusion of cobalt chloride into the median nerve (Fig. 2*a*). In the adult these two arborisations consist of repeated branching into the paths of the descending retinula cells. Stains of earlier instars reveal a much reduced branching structure. It is interesting to speculate that the apparent contact guidance of second-order cells from the brain into the ocellus may be followed by the advance of long collaterals from the second-order cells into retinula cell bundles through a similar mechanism in which the newly developed retinula axons compete for collaterals. Support for this theory comes from adult brains that occasionally contain a third supernumerary median to lateral cell^{5,6}. In this circumstance the arborisations of each individual cell are simpler than in the normally innervated retina (Fig. 2*a* and *b*). Manipulative experiments are in progress to confirm these observations and to determine what other parameters may affect the form of the second-order cell

patterns. Further structural details of ocellar development are to be discussed elsewhere.

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Plasmodium falciparum gametocytogenesis *in vitro*

THE mechanism of sexual reproduction among malaria parasites is coming under increasing scrutiny. Gametogenesis is being unravelled by electron microscopy¹ and by kinetic studies². Gametocytogenesis on the other hand remains poorly understood in spite of earlier work^{3–5}. The development of *Plasmodium falciparum* gametocytes is particularly intriguing because in man they develop in the deep tissue spaces, especially the spleen and bone marrow. The immature stages only rarely appear in the peripheral blood and as a result have escaped detailed experimental investigation until now. Furthermore, it has long been suspected that their development is prolonged⁶, taking 8–12 d, although there are suggestions to the contrary^{6,7}. Immature gametocytes of *P. falciparum* have recently been reported in cultures thought to be composed only of asexual parasites⁸. I have used a similar microculture technique which has permitted the development *in vitro* of morphologically mature *P. falciparum* gametocytes and present here some preliminary observations on the process of gametocytogenesis in *P. falciparum* infections.

Parasites were obtained from 27 unselected patients aged 6 months–6 yr. Parasitaemias were measured by standard techniques. Heparinised venous blood was washed once with

medium 199. From each patient cultures were set up composed of medium 199 supplemented with 200 mg% glucose, 30 mg% glutamine, 25% (v/v) foetal calf serum and 2.5% (v/v) of washed packed blood cells. Aliquots (200 µl) of the culture were dispensed into flat-bottomed 6-mm tissue culture microplates (Linbro) and maintained in a CO₂ incubator (5% CO₂ in air) at 37 °C. The medium was replaced every 2 d. The culture was sampled each day by taking one 200-µl aliquot for the preparation of blood smears.

Table 2. Development *in vitro* of gametocytes from parasites in samples of blood taken from patients on succeeding days

Patient	Days on which blood was taken	Asexual parasites present in blood taken from patient (per 10 ⁵ red cells)	Immature gametocytes grown <i>in vitro</i> from the blood samples (per 10 ⁵ red cells)
AJ	0	231	26
	2	610	222
YD	0	480	20
	2	44	5
JG	0	577	2
	1	4,318	1
	2	1,095	2
	3	3,100	6
MG	0	2,727	3
	1	2,040	13
	2	238	1
	3	40	6
	6	154	5
	10	1,194	275
	14	1,926	53

Gametocytes developed in 80% of cultures; that is in 22 taken from 27 different subjects. The cultured gametocytes were classified into stages of development⁹ based on the detailed morphological descriptions made by earlier workers from post-mortem specimens and tissue biopsies^{4,10}. As a result a sequential pattern of development for the growing gametocytes was demonstrated (Fig. 1). The significance and relevance of this morphological pattern to the developmental biology of *P. falciparum* gametocytes is under study. In man the gametocytes of *P. falciparum* first appear in the peripheral blood about 10 d after the asexual parasites^{3–5}. The cultures *in vitro* give direct confirmation that the 10 d are occupied by the progressive development of the gametocytes rather than by an inductive process and a shorter period of development⁷. The mean culture life was 8.8 d during which all the immature gametocytes grew to at

Table 1 Growth and multiplication of trophozoites, and the development of gametocytes of *Plasmodium falciparum* *in vitro*

Patient	Time (h) since culture started	No. of parasites per 10 ⁵ red cells	Rings	Small trophozoites	Large trophozoites	Early schizonts	Mature schizonts	Gametocytes*			Dead parasites (asexual and sexual)
								I	II	III	
SB	0	1,450	72	28	—	—	—	—	—	—	—
	24	1,210	—	14	12	26	12	36	—	—	—
	48	5,190	62	29	—	2	1	3	3	—	—
	72	4,650	21	39	7	—	—	4	8	4	13
FC	0	900	100	—	—	—	—	—	—	—	—
	24	850	8	24	26	18	—	14	—	—	—
	48	3,520	30	52	4	1	1	30	—	—	16
	72	4,364	16	64	7	2	—	30	24	—	34
MG	0	1,926	40	40	20	—	—	—	—	—	—
	24	1,889	—	18	60	20	—	2	—	—	—
	48	5,632	40	22	8	8	16	—	4	—	2
	72	7,302	18	50	16	6	—	—	—	6	4
AJ	0	610	40	50	10	—	—	—	—	—	—
	24	880	6	22	22	36	6	8	—	—	—
	48	2,700	40	44	—	13	2	—	1	—	—
	72	4,760	22	32	21	8	—	6	5	1	5
	96	4,770	9	9	25	4	—	—	5	3	45

*Gametocytes classified according to Hawking *et al.*⁹ (see Fig. 1).

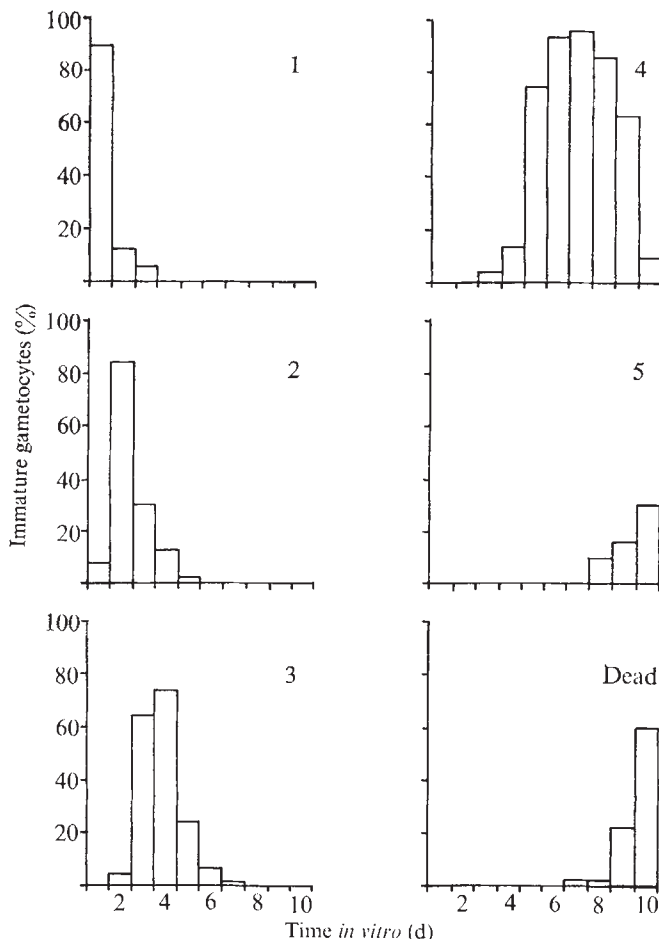


Fig. 1 Sequential development of immature gametocytes of *Plasmodium falciparum* grown *in vitro*. The gametocytes were classified into stages of development according to the outline of Field and Shute⁴ and modified by Hawking *et al.*⁹; 5 developmental stages were recognised. (1) Rounded parasite, not filling the red cell; (2) parasite irregular shape, slightly elongated, not filling the red cell; (3) parasite elongated, red cell slightly distorted; (4) parasite elongated, red cell very distorted; (5) mature crescentic gametocyte. Dead gametocytes were identified as parasites within red cells that were either elongate and very densely stained or were rounded up and lightly stained. These probably represent gametocytes that died at different stages of their development.

least the 4th stage of development and some cultures produced up to 32% of morphologically mature gametocytes. There is known to be an interval of 1–2 d between the development of morphological maturity and the attainment of functional maturity¹¹. It seems that the longevity of the cultures, limited so far by the ability of red cells to survive prolonged periods *in vitro* at 37 °C has prohibited the development *in vitro* of functionally mature gametocytes.

The asexual parasites in the original sample of blood grew *in vitro* and after 36–48 h significant reinvasion of erythrocytes by the liberated merozoites was obtained (mean multiplication rate of 4.8 times). During the first 24 h *in vitro* no increase in parasite numbers was recorded and yet immature gametocytes were detected (Table 1). It is clear therefore that very young gametocytes, as newly formed rings, invade the peripheral blood along with the young asexual rings that come from the same population of schizonts. Both populations then withdraw to the deep tissues to complete their development, the asexual parasites taking a further 18–24 h and the gametocytes 8–9 d. Phillips *et al.*⁸ have further reported that ring-stage parasites derived from the peripheral blood and cultured *in vitro* to the schizont stage can subsequently give rise to both sexual and asexual lines of *P. falciparum* parasites *in vitro*.

Gametocytogenesis in *P. falciparum* infections within

adult humans has been positively correlated with the onset of clinical malaria rather than simply with increasing asexual infection⁷. The event is probably not therefore the consequence of an intrinsic process that gives an increasing probability of detecting gametocytes from a higher compared to a lower asexual infection. Only 59% of my cultures started from patients with asexual parasites alone in their peripheral blood produced developing gametocytes *in vitro*, compared with 100% of the cultures taken from patients with an infection that had already started to produce mature gametocytes *in vivo*. These results confirm that gametocytes are not produced immediately the blood infection begins, rather there is a definite point in the infection when gametocytogenesis starts for the first time. Once gametocyte production has begun from the persisting asexual infection, however, then each successive schizogony produces further gametocytes. Four patients studied on more than one occasion supported this view (Table 2). It is suggested that there is a trigger for the start of gametocytogenesis but once the process is established within an infection then gametocyte production becomes an intrinsic part of the life cycle.

The culture technique described has opened a new area of the life cycle of *P. falciparum* to detailed investigation. The preliminary observations confirm the prolonged development of *P. falciparum* gametocytes. They also show that once gametocyte production has begun each schizogony is responsible for fostering sexual reproduction the elements of which make a temporary appearance in the peripheral blood before withdrawing to the deep tissues.

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Cell surface design

CONSIDERATIONS of membrane fluidity (see refs 1–5) have often involved schematic representations of the cell plasma membrane and underlying cytoskeletal structures^{3–5}. But, although intended to show how microtubules, microfilaments and so on might interfere with the mobility of membrane components, such models have not been drawn to scale. Usually microtubules and microfilaments seem much smaller than the plasma membrane, whereas the reverse is true. Thus, it has not been possible to evaluate fully the relative importance of plasma membrane and cytoskeleton in influencing cell surface dynamics. I have therefore tried to draw these various elements to scale. Since the data come from heterogeneous sources (fibroblasts, muscle cells, amoeba, plant cells, epithelial cells and lymphocytes) it is clear that the organisation of the various structures represented here can only be hypothetical and does not correspond to a known structure in a given cell type.

Figure 1 is a representation of such a structure; it shows a section of microvillus (MV), with actin microfilaments (MF), α -actinin molecules (α -A), myosin molecules arranged as a filament (MM); the cortical cytoplasm with microtubules (MT) and their cross bridges (c-b), the plasma-membrane (PM) with extrinsic and intrinsic proteins (drawn

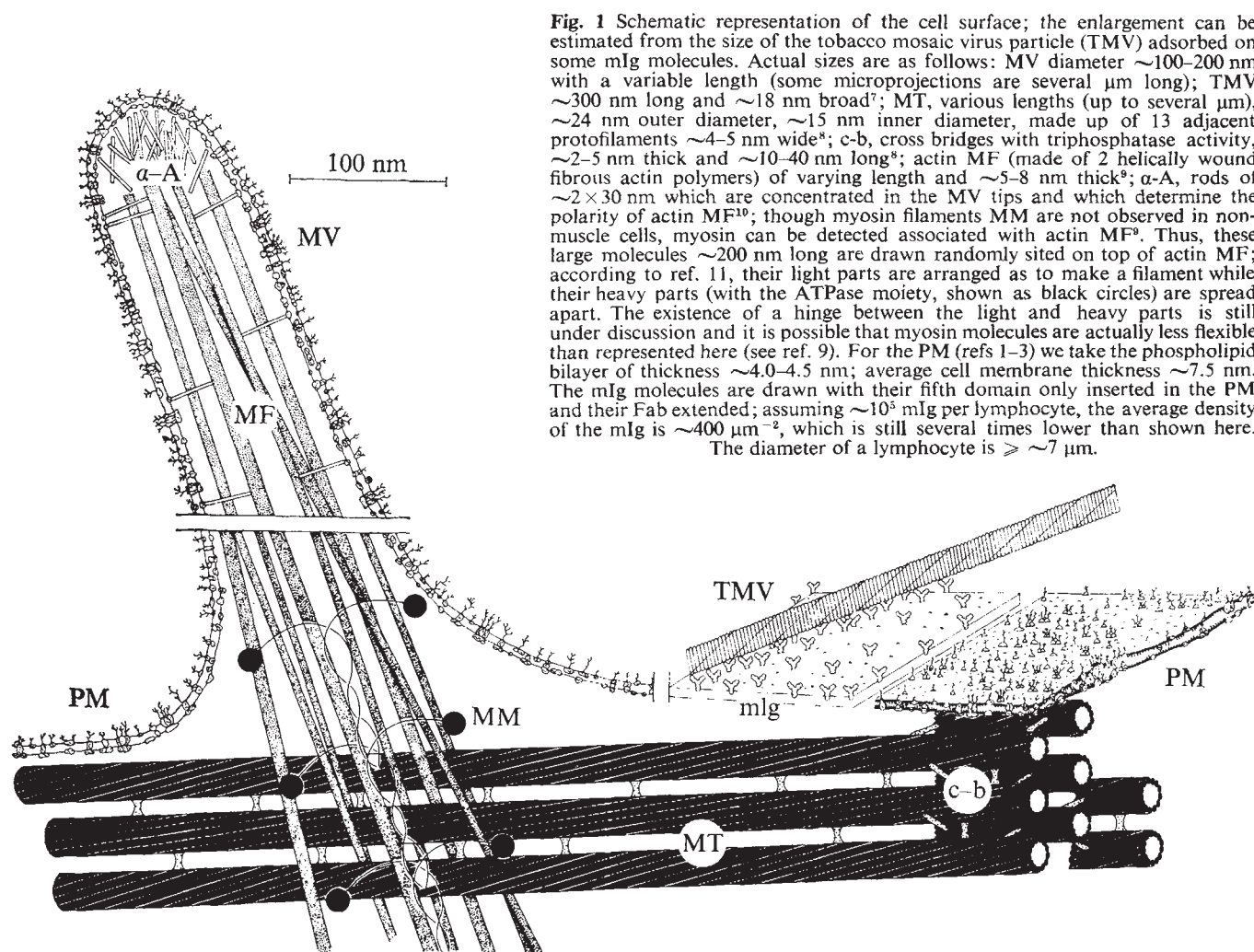


Fig. 1 Schematic representation of the cell surface; the enlargement can be estimated from the size of the tobacco mosaic virus particle (TMV) adsorbed on some mIg molecules. Actual sizes are as follows: MV diameter $\sim 100\text{--}200\text{ nm}$ with a variable length (some microprojections are several μm long); TMV $\sim 300\text{ nm}$ long and $\sim 18\text{ nm}$ broad⁷; MT, various lengths (up to several μm), $\sim 24\text{ nm}$ outer diameter, $\sim 15\text{ nm}$ inner diameter, made up of 13 adjacent protofilaments $\sim 4\text{--}5\text{ nm}$ wide⁸; c-b, cross bridges with triphosphatase activity, $\sim 2\text{--}5\text{ nm}$ thick and $\sim 10\text{--}40\text{ nm}$ long⁹; actin MF (made of 2 helically wound fibrous actin polymers) of varying length and $\sim 5\text{--}8\text{ nm}$ thick⁹; $\alpha\text{-A}$, rods of $\sim 2 \times 30\text{ nm}$ which are concentrated in the MV tips and which determine the polarity of actin MF¹⁰; though myosin filaments MM are not observed in non-muscle cells, myosin can be detected associated with actin MF⁹. Thus, these large molecules $\sim 200\text{ nm}$ long are drawn randomly sited on top of actin MF; according to ref. 11, their light parts (with the ATPase moiety, shown as black circles) are spread apart. The existence of a hinge between the light and heavy parts is still under discussion and it is possible that myosin molecules are actually less flexible than represented here (see ref. 9). For the PM (refs 1–3) we take the phospholipid bilayer of thickness $\sim 4.0\text{--}4.5\text{ nm}$; average cell membrane thickness $\sim 7.5\text{ nm}$. The mIg molecules are drawn with their fifth domain only inserted in the PM and their Fab extended; assuming $\sim 10^5$ mIg per lymphocyte, the average density of the mIg is $\sim 400\text{ }\mu\text{m}^{-2}$, which is still several times lower than shown here. The diameter of a lymphocyte is $\geq \sim 7\text{ }\mu\text{m}$.

to the typical Singer–Nicolson pattern) and with an area which shows some receptor immunoglobulins (mIg) and belongs to a lymphocyte surface (for a complete analysis, see ref. 6).

This model is very different from all those so far published. For instance, it now seems impossible that several microfilaments or microtubules could be linked to single plasma-membrane proteins, as has usually been postulated. On the contrary, several membrane proteins might be linked to single microfilaments or microtubules, by means of elements such as α -actinin molecules or microtubule cross bridges. Furthermore, it seems unlikely that phospholipid flow is the major force driving the redistribution of membrane components in processes such as ‘capping’, on lymphocytes as well as on other cells. More likely, contractile microfilaments are involved in such gross cell surface rearrangements, the principal role of microtubules being more static. I have suggested elsewhere⁶ an hypothesis of how the antagonistic functions of microfilaments and microtubules might be co-ordinated. My idea is that they would confer to the cortical cytoplasm of the cell properties like those of a thixotropic gel.

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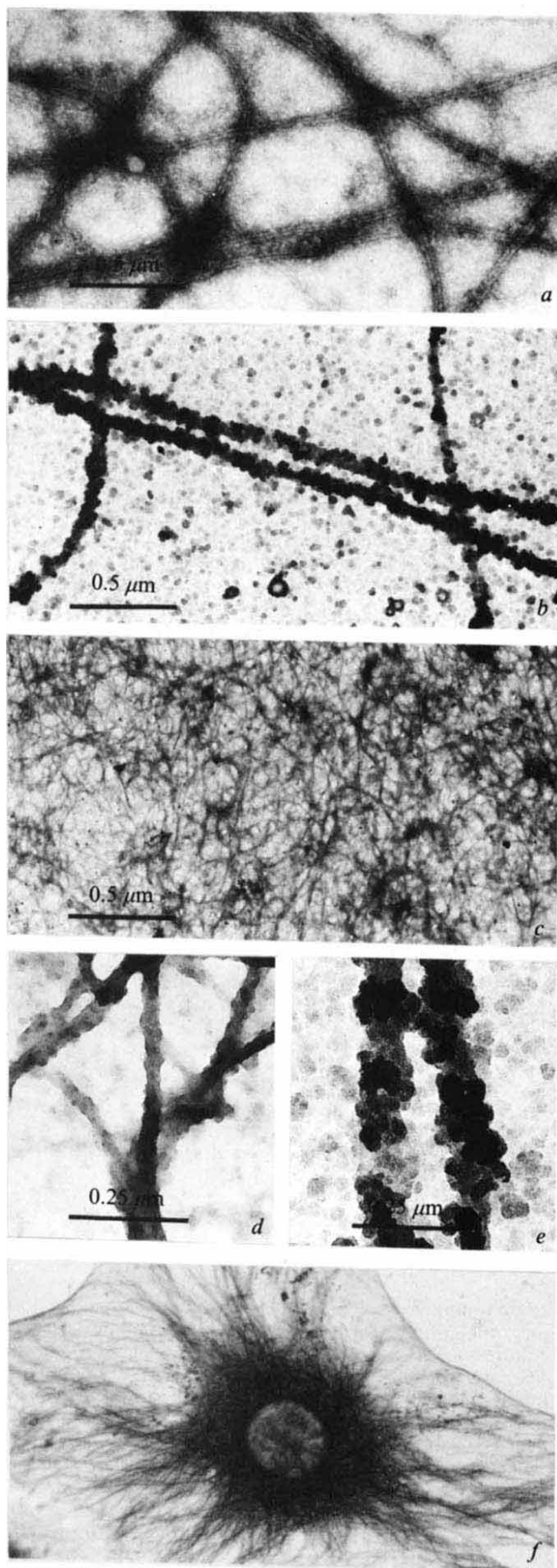
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Immunoperoxidase visualisation of microtubules and microtubular proteins

MICROTUBULES are ubiquitous eukaryotic organelles, formed by polymerised tubulin and presumably some additional proteins, which are involved in a wide variety of cell functions (for review see refs 1 and 2). Evidence has been presented for the existence of a dynamic equilibrium between polymerised and non-polymerised microtubular proteins³. Recently immunofluorescence of cultured cells demonstrated a fine cytoplasmic network that was believed to correspond to cytoplasmic microtubules. However, no direct evidence was presented that the antibodies stain individual microtubules, the formaldehyde fixation that was used is not known to preserve microtubules intact and immunofluorescence does facilitate comparison of the total picture of the microtubular apparatus with its appearance in histological and ultrastructural sections (horizontally or vertically) through the same cell. Moreover, the inadequate resolution of the light microscope does not allow an accurate localisation of the



non-polymerised tubulin and the study of its interaction with various cellular organelles. We have therefore developed an immunochemical technique described here that could simultaneously be used for the localisation of microtubules and tubulin in whole cells and in histological and ultrastructural sections, after adequate fixation with glutaraldehyde.

The unlabelled peroxidase-anti-peroxidase (PAP) method of Sternberger⁶ which had previously been adapted for the localisation of neurophysins⁷ seemed to fulfil the necessary criteria. In this method, the antibody associated with tubulin is allowed to react with an excess of an anti-immunoglobulin (as in the fluorescent "sandwich" method), and then, in turn, with a soluble complex of peroxidase and its antibody. The latter complex acts as an antigen and combines with the free antibody sites and may then be revealed by chemical reactions that lead to the deposition of osmium.

Before attempting to use whole cells, we have assessed whether microtubules polymerised in a cell-free system can be stained specifically with this method.

Figure 1b shows that individual microtubules polymerised *in vitro* and fixed with glutaraldehyde can, indeed, be stained by the PAP method. In the electron microscope

Fig. 1 *a*, Microtubules polymerised *in vitro* and negatively stained with uranyl acetate. Tubulin was prepared from rat brain homogenate and polymerised as described previously⁸. One drop of the solution was placed on a nickel grid coated with Formvar. After 1 min the grid was rinsed with a few drops of hexylene glycol 1 M and fixed for 1 min in 2.5% glutaraldehyde at room temperature. After rinsing with sodium cacodylate 0.1 M the grids were placed for 30 s on a drop of 0.5% uranyl acetate, then drained and dried. *b*, Microtubules polymerised *in vitro* and stained with the PAP-technique. Nickel grids with microtubules obtained and fixed as described above were placed on a drop of normal goat serum diluted 1/30 in Tris-buffered saline (TBS), pH 7.4 for 10 min. Then the grids were washed for 10 min by flotation on TBS on a rocking table. Hereafter the grids were placed for 30 min on a drop of TBS containing a 1/3,200 dilution of antibody solution II (see Table 1) and 1% goat serum. After washing in TBS for 20 min the grids were placed for 30 min on a drop of goat antiserum to rabbit immunoglobulins (1/20 in TBS) (GAR/Ig Nordic Pharmaceuticals and Diagnostics), and subsequently washed with TBS. Thereafter the grids were placed for 30 min on a drop of TBS containing a 1/50 dilution of the PAP complex and 1% goat serum, and subsequently washed. This was followed by the reaction with 3,3'-diaminobenzidine (DAB) for 7 min, staining with osmium tetroxide 2% on 0.05 M veronal acetate, washing with veronal acetate and drying. The reaction with DAB was carried out according to Weir *et al.*¹¹. Note the accumulation of darkly stained PAP complexes on the microtubules and the diffuse layer of PAP complexes between them. *c*, Actin filaments polymerised *in vitro* according to the method of Carsten¹². The fibres were adsorbed on to nickel grids covered with Formvar, fixed with glutaraldehyde and stained with the PAP method using the antibody dilution, in an identical fashion as described for *b*. Note the complete absence of PAP complexes from the filaments. *d*, Microtubules polymerised *in vitro* and stained with the PAP method as described above with omission of the antibody solution II. Note the complete absence of PAP complexes from the microtubules and from the spaces between them. The enlargement of the microtubules (± 35 nm instead of ± 24 nm) is probably due to the rehydration and the coating with normal goat serum. *e*, Part of *b* enlarged to the same magnification as *f*. Untransformed mouse embryonal cell in culture (MO)¹³ stained with the PAP-technique. Cells, grown on cover slips, were fixed in 0.25% glutaraldehyde in 0.1 M sodium cacodylate pH 7.4, for 5 min. After washing in the same buffer (30 min), they were postfixed with a series of acetone 50% $\rightarrow$ 100% $\rightarrow$ 50% at 4°C; and placed in TBS for 15 min; brief washing in TBS; 1/800 antibody dilution (no. II, Table 1) in TBS $\times$ 1% normal goat serum, 30 min; extensive washing as before; 1/20 GAR, 30 min; extensive washing as before; 1/300 PAP in TBS containing 1% normal goat serum, 30 min; extensive washing in TBS. The reaction with DAB was the same as in Fig. 1b. The reaction product was stained with osmium tetroxide (2% in veronal acetate) for 10 min at 4°C. After washing, the cover slips were mounted in Fluormount with the cells downwards. Note the dense cytoplasmic network and the absence of nuclear staining, except for pronounced staining of the nuclear membrane ($\times 500$).

individual microtubules appear as heavily stained arrays of PAP complexes with a diameter of ± 100 nm. This size can be expected in view of the diameter of microtubules (± 24 nm), and of the PAP complex (± 20 nm) linked to the microtubules by a double layer of immunoglobulins. Because of this enlargement the labelled microtubules are clearly visible when the grids are examined with the light microscope. Fixation with formaldehyde (3.5%; 5 min) results in a distortion of the microtubules and a loss of the protofilamentous substructure at the ultrastructural level. But these structures still bind the immune complex. After glutaraldehyde or formaldehyde fixation PAP complexes are evenly distributed in the open spaces between the microtubules (Fig. 1b). The staining pattern is identical when microtubules are polymerised directly from a 100,000g supernatant from homogenised rat or rabbit brain⁸ or when microtubules are prepared from a tubulin solution that is enriched by two polymerisation-depolymerisation steps⁹.

Table 1 Results obtained with different antibody solutions and different substrates

Substrate	Antibody solution	Dilution	Staining
Microtubules	II purified γ -G	1/800–1/50,000	+
	Immune rabbit serum	1/100,000	—
Microtubules	I γ -G	1/800–1/6,400	+
	Immune rabbit serum	1/12,500	—
Actin	I	1/3,200	—
Collagen	I	1/3,200	—
Microtubules	III	1/800–1/3,200	+
	Antiserum against purified tubulin		
Microtubules	IV RAB/Alb	1/800	—
Microtubules	V adsorbed γ -G normal rabbit serum	1/800	—

Antibody solution I was prepared by injecting rat brain tubulin, purified by two polymerisation-depolymerisation steps⁹, homogenised with Freund's complete adjuvant. The immunoglobulin serum fraction was obtained by two precipitations with sodium sulphate. The protein concentration was 36.6 mg ml⁻¹. Antibody solution II was derived from solution I through further purification by affinity chromatography on Sepharose-4B to which the antigen was coupled. The protein concentration was 5 mg ml⁻¹. Antibody solution III is a crude antiserum raised against SDS-gel purified tubulin. Antibody solution IV is a rabbit anti-bovine serum albumin antiserum (RAB/Alb Nordic Pharmaceuticals and Diagnostics, Antwerp, Belgium). Antibody solution V is an immunoglobulin G fraction from normal rabbit serum adsorbed on to a column containing Sepharose-4B to which tubulin was coupled. The protein concentration was 15 mg ml⁻¹.

When rabbit muscle actin filaments, or rat skin collagen fibres¹⁰, polymerised *in vitro*, are subjected to an identical procedure, they remain completely unstained (Fig. 1c). Neither is any background visible, as is the case with formvar-coated grids that were not covered with any protein. Microtubule staining is also completely absent if one or more of the immune reagents is omitted (Fig. 1d). When rabbit anti-bovine serum albumin IgG or purified rabbit γ -globulins, previously absorbed on to a column of Sepharose 4B to which tubulin was coupled, are used the microtubules are not stained. Neither is any background staining visible. The results that were obtained with various antibody solutions on different substrates are given in Table 1.

Preliminary results, using the fixation procedure of Brinkley *et al.*⁴ but the PAP method instead of immunofluorescence showed that a fine cytoplasmic network and the mitotic spindle can be visualised in cultured cells at the light microscopic level. This resembles the immunofluorescent picture in all respects. Moreover, a similar network is visible after fixation with glutaraldehyde (Fig. 1f). The network seems to consist of more individual lines that have a greater definition than after formaldehyde fixation. Finally, semithin

(1 μ m) and thin sections (0.07 μ m) can be made of the same cells after embedding them in Epon.

In semithin sections the cytoplasmic network is visible either sectioned longitudinally or transversely.

At the ultrastructural level, both cytoplasmic and mitotic spindle microtubules are seen to be covered with stained PAP complexes, that are filling the clear zone normally present around them.

The cytoplasm contains evenly distributed PAP complexes. The mitochondria, the lumen of the endoplasmic reticulum, Golgi cisternae and vacuoles, and the bundles of microfilaments are completely unstained. The nuclear periphery, with the exception of the pores, shows pronounced staining. The nuclear content is negative. These preliminary observations are, however, subject to further investigation.

In conclusion, we have shown that it is possible to stain microtubules and most probably microtubular proteins in other configurations, with the PAP method using antibodies against purified rat brain tubulin. This method does not stain other fibrillar proteins such as actin or collagen.

This method is applicable at the light microscopic and ultrastructural level and provides a way of exploring the distribution of microtubules, tubulin and associated proteins and their interaction with other cellular components.

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Barbiturate reversal of amino acid antagonism produced by convulsant agents

THE hypnotic and anticonvulsant properties of pentobarbitone and related barbiturates may be closely associated with the actions of γ -aminobutyric acid (GABA)¹⁻³, an inhibitory transmitter at both pre- and postsynaptic receptors in the mammalian central nervous system⁴. Pentobarbitone prolongs pre-⁵ and postsynaptic³ inhibition as well as hyperpolarising central neurones^{2,3}. Although it has been suggested that pentobarbitone prolongs synaptic inhibition by delaying the removal of GABA from the synaptic cleft^{3,6} a direct action of pentobarbitone on GABA receptors cannot be excluded². We have explored this possibility both in the rat isolated sympathetic superior cervical ganglia preparation, which possesses GABA receptors analogous to those in the mammalian brain⁷, and *in vivo* on single neurones in the rat brainstem. In these experiments pentobarbitone, in amounts which produced no apparent GABA-mimetic effects, reversed the actions of bicuculline methochloride (BMC), a selective GABA antagonist.

Ganglia were isolated from Wistar rats under urethane anaesthesia and desheathed before superfusion with Krebs' solution (containing 2.6 μ M hyoscine) as described previously⁸. Surface potentials were monitored continuously through Ag⁺/AgCl electrodes in contact with the ganglion body and postganglionic trunk and connected across a Servoscribe 1-s potentiometric recorder.

The addition of GABA, at concentrations greater than 1 μ M, to the solution superfusing the ganglia produced a dose-dependent depolarisation. Pentobarbitone also depolarised ganglia but only at much higher concentrations. The threshold concentration was in excess of 80 μ M. Responses to pentobarbitone were less susceptible to the effects of BMC than were responses to equally effective doses of GABA.

Application of pentobarbitone during superfusion with BMC reversed the action of this GABA antagonist. An example of this reversal is shown in Fig. 1. Responses to GABA (30 μ M) were reduced approximately 50% in the presence of BMC (14 μ M). Addition of pentobarbitone in increasing concentrations reversed the effect of BMC. At this concentration of BMC the maximum reversal occurred generally at 80 μ M pentobarbitone. The effect of pentobarbitone was rapid in the onset, as well as offset; within 20 min following its removal responses to GABA were again reduced to the original antagonised level.

Pentobarbitone (80 μ M) applied in the absence of BMC (Fig. 1) generally did not produce responses to GABA administered at concentrations up to 30 μ M. But responses to larger concentrations of GABA were always markedly reduced. In spite of this, responses obtained to these concentrations of GABA, in the presence of both pentobarbitone and BMC, were always greater than in the presence of either substance alone.

The antagonism of responses to GABA produced by other substances, picrotoxin, tetramethylenedisulphotetramine and isopropyl bicyclopophosphate was also reversed by pentobarbitone whereas the antagonism of depolarising responses to carbachol (33 μ M), produced by hexamethonium (75 μ M), was not. In addition the non-selective depression of responses to GABA produced by strychnine⁷ (73 μ M) was reversed by pentobarbitone but the similar depression of responses to carbachol, produced by the same concentration of strychnine, was not reversed.

Other barbiturates also reversed the effects of the BMC. However, of those so far tested, only quinalbarbitone was found to be more potent (2-3 times) than pentobarbitone. The isomer of pentobarbitone, amylobarbitone, and the sulphur derivative thiopentone had similar activity to pentobarbitone whereas phenobarbitone was about 5% the potency.

This investigation was extended to *in vivo* experiments

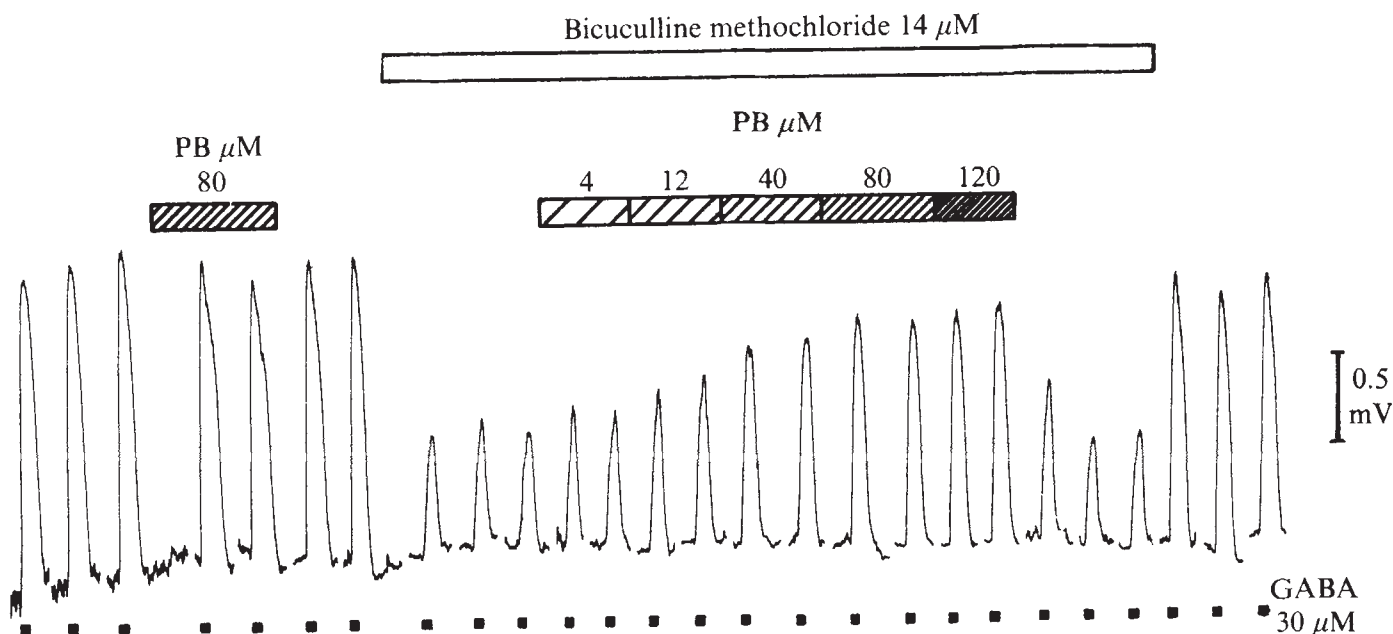


Fig. 1 Reversal by pentobarbitone of the antagonism of depolarising responses to GABA produced by bicuculline methochloride in an isolated superior cervical ganglion of the rat. The ganglion was superfused with Krebs' solution at 1 ml min⁻¹ and the surface potential measured as described previously^{7,8}. GABA (30 μ M) was applied for 1-min periods, indicated by the solid bars below the record, at intervals of 13 min. The recorder paper drive was switched off in between responses for periods of approximately 8 min (gaps in record). Pentobarbitone (PB, 4-120 μ M) and bicuculline methochloride (14 μ M) were present in the superfusing solution during the periods shown. Note that (1) pentobarbitone (80 μ M) applied in the absence of bicuculline methochloride neither depolarised the ganglion nor markedly altered responses to GABA, and that (2) increasing concentrations of pentobarbitone reversed the depression of responses to GABA produced by bicuculline methochloride.

performed on six adult albino rats (200–250 g) anaesthetised with intraperitoneal urethane (1.4 g kg^{-1}) and prepared for brainstem recording by the method of Bradley and Dray⁹.

Multibarrelled glass micropipettes were used to record extracellular action potentials from single spontaneously active neurones in the medulla and to administer substances close to them by electrophoresis. Micropipettes were filled immediately before use by a glass-fibre method or by centrifugation. The recording barrel contained 3 M sodium chloride. Another barrel containing 1 M sodium chloride was used to balance the net current at the electrode tip to 0 or to test for electrophoretic current effects. Other barrels contained freshly prepared solutions of GABA,

kg^{-1} , four cells). Pentobarbitone also reversed the non-specific antagonism of glycine by BMC on every occasion. The effects of pentobarbitone were never accompanied by significant changes in background firing and the antagonism of GABA by BMC was rapidly restored after the end of the pentobarbitone administration (Fig. 2). This antagonism of GABA could also be restored during a pentobarbitone administration by increasing the ejecting current of BMC.

Further tests with pentobarbitone alone, administered to the same neurones (six cells) on which a reversal of BMC antagonism had already been demonstrated, did not reveal any significant potentiation of submaximal responses to GABA or glycine. In fact, on occasions the responses to

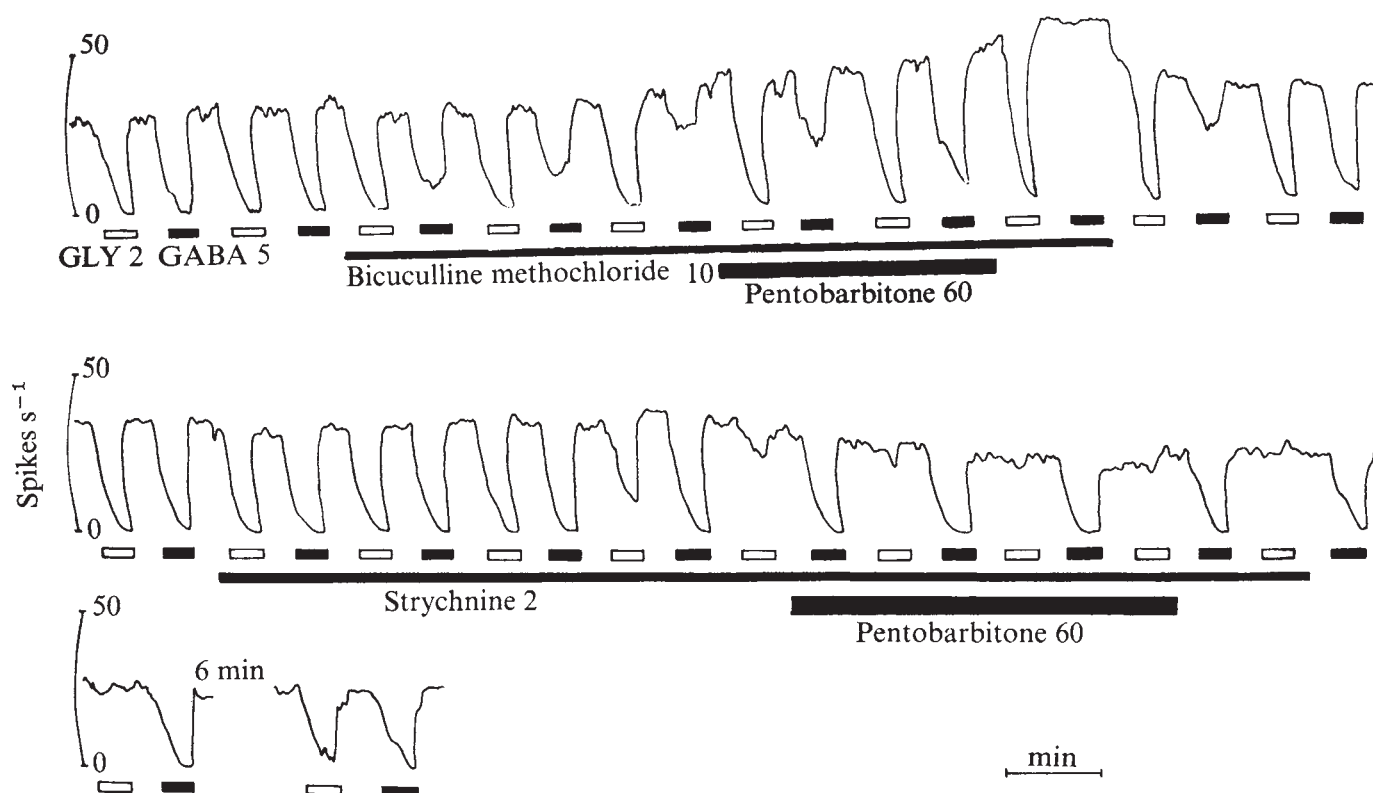


Fig. 2 Continuous ratemeter records of a spontaneously active neurone in the medulla showing the reversal by pentobarbitone of a selective antagonism of GABA by bicuculline methochloride. The GABA block by BMC was restored after the termination of the pentobarbitone administration. Strychnine selectively abolished the response to glycine on the same cell but this effect was not reversed by pentobarbitone. All expelling currents are indicated in nA.

0.2 M (pH 3.5); glycine, 0.2 M (pH 3.5); bicuculline methochloride, 5 mM (pH 3.5); strychnine, 5 mM (pH 3.5); pentobarbitone sodium, 20 mM (pH 9.5). Action potentials were recorded and displayed using conventional techniques.

Reproducible responses to GABA and glycine were obtained during consecutive applications of these compounds during a fixed time sequence. A continuous administration of BMC (10–20 nA) reduced the responses to GABA in all 19 neurones tested an example of which is shown in Fig. 2. In four of these cells responses to glycine were also partially reduced.

Pentobarbitone (40–80 nA, mean 56.6 nA; 1–6 min, mean 3.7 min) ejected from an adjacent barrel of the same micropipette during the continued administration of BMC partially or completely reversed the GABA antagonistic effect of BMC (Fig. 2). The reversal occurred in all neurones tested (26 tests on 19 neurones) and was also seen following intravenous administration of thiopentone (1 mg

GABA and glycine were reduced by the same current of pentobarbitone previously shown to reverse the antagonism produced by BMC. Larger currents (>100 nA) of pentobarbitone produced marked depression of spontaneous firing.

Strychnine (2–5 nA) reversibly reduced the effects of glycine on 13 neurones and with larger ejecting currents (10–15 nA) additional reduction of the responses to GABA was observed (two cells). All tests with strychnine were performed on neurones which had also been tested before with BMC. The additional administration of pentobarbitone (30–70 nA; mean 64 nA; 2–13 min, mean 5.8 min) during a partial (50%) or complete suppression of glycine by strychnine, reversed strychnine antagonism in only five neurones. In most neurones strychnine antagonism of glycine was not affected although the amounts of pentobarbitone were in excess of those which had reversed BMC antagonism of GABA on the same cells. On the two cells where a non-

selective strychnine antagonism of GABA was observed, pentobarbitone readily and reversibly restored the GABA responses.

At present it is difficult to understand the mechanism by which barbiturates reverse the effect of BMC or other GABA antagonists. It might be argued that the inhibition of GABA uptake produced by pentobarbitone *in vitro*⁶ could account for the reversal. Uptake inhibition would increase the concentration of GABA in the vicinity of the receptors which might then overcome the competitive antagonism produced by BMC. But, uptake inhibition would also be expected to potentiate the responses to GABA even in the absence of BMC (compare the potentiating effect of nipecotic acid—ref. 10). However, no potentiation of responses to GABA was observed when pentobarbitone was administered in amounts which were subsequently shown to reverse the effect of BMC. In addition the presence of 3 mM nipecotic acid, which reduced ganglionic uptake of ³H-GABA (0.1 μ M) by more than 90%, did not affect the ability of pentobarbitone to reverse the action of BMC. An alternative explanation might be that GABA and BMC bind to the cell surface at different sites. If BMC binds at a site adjacent to the GABA site such that, when present, it prevents access of GABA to the receptor not because of preferential binding to the receptor but by either occluding it, or by distorting it. If pentobarbitone has an affinity for the antagonist binding site it could displace the BMC without itself affecting the receptor. GABA could then regain access to the receptor.

The possibility of a difference in agonist and antagonist binding sites is not without precedent. Snyder and Young¹¹⁻¹³ have suggested that glycine and strychnine bind to two separate but mutually interacting sites on synaptic membrane preparations of spinal cord and brainstem. They suggest that the antagonist binding site may, in fact, be associated with the ionic conductance mechanism for chloride whereas the agonist, glycine, interacts with the glycine recognition site. Snyder and his group¹⁴ have also demonstrated GABA receptor binding in similar preparations. However, as yet there is no evidence of separate binding sites for BMC and GABA. Interestingly, in this same study it was shown that pentobarbitone does not affect GABA binding. It remains to be seen whether pentobarbitone affects the binding of BMC in the same preparation.

The barbiturates are particularly useful in the treatment of convulsant poisoning^{15,16}. The present observations suggest that this action may be related to an effect at sites associated with inhibitory amino acid receptors.

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Digynic triploidy after superovulation in mice

SUEROVULATION through the administration of exogenous gonadotropins¹ has been widely used in mice for experimental purposes, and cytogenetic evidence has shown^{2,4} that the technique does not affect adversely the chromosome constitution of either released ova or zygotes. We have noticed, however, that the incidence of triploidy increased considerably among zygotes obtained through superovulation followed by natural mating in adult A-strain mice³. We have therefore investigated the origin of the extra genome in such embryos.

Six- to 8-week-old A/He females received 10 IU of pregnant mare serum (PMS, Schering) at 16.00 h and 10 IU of human chorionic gonadotropin (HCG, Schering) 44 h later. They were allowed to mate with CBA-T₆T₆ males after the second injection. Spontaneously ovulating and mated females served as controls. Eggs at the pronuclear stage were flushed from the oviduct early in the afternoon of the day when a copulation plug was found. Postimplantation embryos were dissected at 7.5 d of gestation from superovulated females, and at 6.5 or 7.5 d from control females. By changing the interval between HCG injection and death, we recovered preimplantation embryos from the eight-cell to the late blastocyst stage.

Totals of 430 and 1,423 viable embryos were obtained from 20 superovulated and 129 control females. Chromosomes were analysed in 421 and 1,406 cases, respectively (Table 1). Failure in nine and 17 embryos was due either to accidental loss of specimens or to the absence of metaphase cells. The frequency of diploid embryos was significantly lower in the superovulated than in the control group, the difference being ascribed to the fivefold increase in the incidence of digynic triploidy (Fig. 1). The frequency distribution of the two possible sex chromosome complements in digynic triploidy was 22XXX:25XXY:5? in the control and 30XXX:45XXY:6? in the superovulated group. The ratio in diandric triploidy was 4XXX:6XXY:1XYY in the control and 1XXY:1XYY in the experimental group, implying apparently reduced viability of XYY zygotes, as suggested in man⁵. Except for this specific class, digynic and diandric embryos, being chromosomally balanced, seemed to be of equal viability. It is, however, possible that more diandric eggs were lost early in development than digynic eggs, if dispermy occurred primarily in deteriorating oocytes. The incidence of triploidy (4.5%) in the control group was nearly comparable with that obtained in 'silver' mice. The high incidence was not ascribed to the interstrain cross⁶, for the situation was not appreciably different when strain A males were used. (N.T., unpublished results.)

The increase in digyny after superovulation would be due to suppression of either the first or the second polar body unless exogenous gonadotropins preferentially stimulated tetraploid oocytes. The nature of the polar bodies, and the number and relative size of the pronuclei¹¹, could reveal a disturbance at maturation division, an error at fertilisation or both. Only a small proportion of activated eggs had the first polar body in our preparation, which agrees with previous findings¹¹. Moreover, about one-tenth of the zygotes with a large (male) and a small (female) pronucleus possessed neither the first nor the second polar body. Inasmuch as these usually had excessively flattened cytoplasm, a loss

of the second polar body during preparation probably explains most of them. The two pronuclei were similar in size in a proportion of eggs from the control and the experimental group. Although variability in pronuclear size might account for most of such eggs, diploidy of the female pronucleus could not be ruled out.

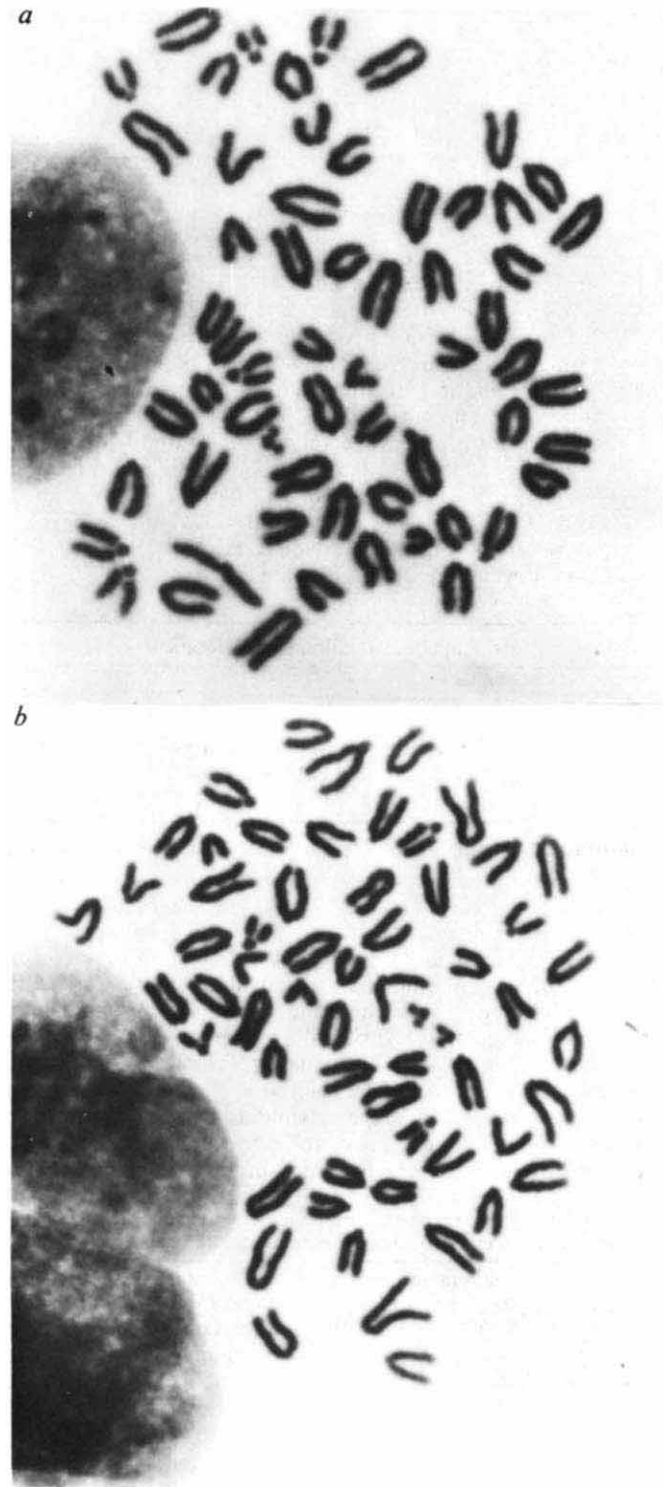


Fig. 1 Triploid metaphases from 7.5-d embryos dissected from A/He females mated with CBA-T₆T₆ males. *a*, Digynic triploidy with a single T₆ marker (arrow) and XXX sex chromosome constitution. *b*, Diandric triploidy with two T₆ markers (arrows) and XYY sex chromosome complex. Dissected embryos were incubated in Eagle's medium supplemented with foetal calf serum (20%) and Colcemid (0.1 $\mu\text{g ml}^{-1}$) for 2 h. Chromosome preparations were made according to the method of Wroblewska and Dyban⁶ with modifications⁷.

A total of 354 of 1,264 superovulated eggs had three pronuclei. Digyny was presumed to occur in 333 and 254 eggs on the basis of the absence of the second polar body and the difference in pronuclear size, respectively. There were 21 diandric (dispermic) eggs estimated on the basis of the retained second polar body, and 49 on the basis of pronuclear size (Fig. 2). Divergence between the two estimates in both classes of eggs may diminish considerably if loss of the second polar body and the variability in pronuclear size are taken into account. The interpretation of our data may be that (1) most triploid embryos identified in later stages derive from zygotes with three pronuclei; (2) digyny outnumbers diandry, and (3) disturbance of the second maturation division is the major cause of digyny. All of the zygotes with three pronuclei found in the control mating were compatible with suppression of the second polar body. Eight zygotes with four pronuclei presumably represented dispermic eggs whose second polar body was suppressed.

Fig. 2 Presumptive triploid zygotes at the pronuclear stage. Two small (female) pronuclei with a single nucleolus in (*a*) suggest digyny due to suppression of the second polar body, and two large (male) pronuclei with several nucleoli in (*b*) suggest dispermy. Possibly the second polar body was lost during preparation.

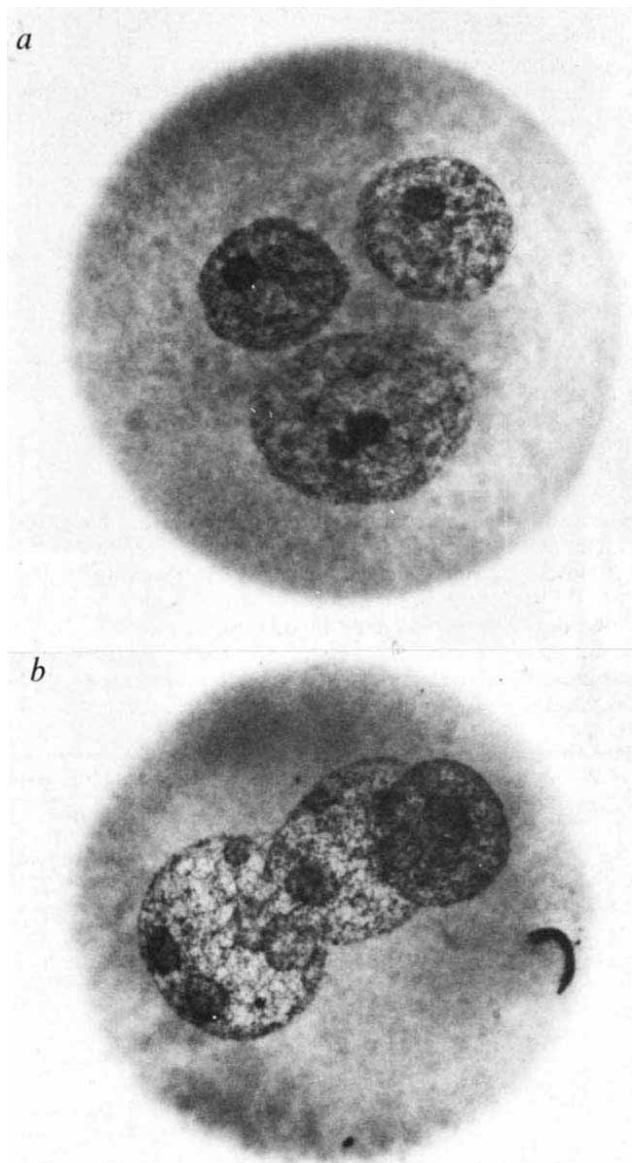


Table 1 Chromosome findings in 6.5–7.5-d embryos from control and superovulated females mated with CBA-T₆T₆ males

	Control	Superovulated	χ^2
No. of litters	129	20	
No. of embryos recovered (mean)	1,423 (10.9)	430 (21.5)	
No. of embryos karyotyped (%)	1,406 (98.8)	421 (97.9)	
2n (%)	1,298 (92.3)	320 (76.0)	83.29†
2n-1 (%)	12* (0.9)	5 (1.2)	0.12
2n+1 (%)	20 (1.4)	4 (1.0)	0.24
Mosaics (%)	9 (0.6)	9 (0.6)	1.50
3n, T ₆ /+/+ (%)	52 (3.7)	81 (19.2)	113.98†
3n, T ₆ /T ₆ /+ (%)	11 (0.8)	2 (0.5)	0.11
4n, T ₆ /T ₆ /+/+ (%)	4 (0.4)	3 (0.7)	0.66
Total heteroploids (%)	108 (7.7)	101 (24.0)	83.29†

*Including one 2n-2.

† $P \leq 0.005$.

The proportion of informative embryos was low at the morula-blastocyst stage because of technical difficulties. We obtained 1,022 informative cases including 219 triploids. As diagnosis of aneuploidy was difficult at this stage, embryos with 38–41 chromosomes were classified as diploid. The incidence of diploid, triploid and tetraploid embryos at pronuclear, morula-blastocyst, and postimplantation stages are compared in Table 2. To make data comparable, genuine diploids, aneuploids and mosaics in the diploid range were combined as a diploid class, and a single 2n/3n mosaic blastocyst was included in a triploid class. No difference was found in the three classes of embryos between the morula-blastocyst and the postimplantation stage, but triploidy was

numbers according to Edwards' formula¹². As Table 3 shows, the ratio between cleavage numbers of triploids and those of diploid sibs was nearly constant, ranging from 0.88 to 0.92 for the stages examined. This may imply that the cell-cycle time was about 10% longer in triploids than in diploids. Extrapolation of this relationship predicts that the cell number of triploid embryos would become half that of diploid sibs at 10th cleavage (about 5.5 d) and a quarter at 20th cleavage (about 10.5 d), though delay might be accelerated after implantation. It should be mentioned, however, that there were substantial variations in developmental potential among individual triploid embryos^{2,13-15}.

Foetal development, investigated extensively in mice

Table 2 Frequency of diploid, triploid and tetraploid embryos from superovulated females in relation to developmental stages

	Pronuclear	Morula-blastocyte	Postimplantation	I	II	III
2n	870	799*	335*	16.20†	12.29†	0.27
3n	354	220†	83	14.87†	12.66†	0.49
4n	8	3	3	0.83	0.04	0.31

I, Between pronuclear and morula-blastocyst stages; II, between pronuclear and postimplantation stages; III, between morula-blastocyst and postimplantation stages.

*Embryos within the diploid range.

†Including a 2n/3n mosaic.

‡ $P \leq 0.005$.

significantly more frequent at the pronuclear stage. Consequently we conclude that a proportion of triploid zygotes was lost in early cleavage stages. Remaining triploids seemed to survive implantation.

The developmental characteristics of triploids before implantation were assessed as follows. Litters were classified into groups according to the average cell numbers of diploid sibs. Average cell numbers were converted into cleavage

after induced ovulation, has revealed substantial mortality during cleavage, about the time of implantation, at mid-pregnancy and at parturition¹⁶⁻¹⁸. The extent to which chromosome imbalances is causally related to embryonic mortality has not been explored yet. Our observation strongly suggests that digynic triploids constitute an appreciable proportion of pre- and postimplantation losses. Possibly the same holds for mice of other strains, even if

Table 3 Cell and cleavage numbers in diploid and triploid preimplantation embryos from superovulated females

Cell numbers $\pm$ s.e.m.	(No. of embryos)	Cleavage numbers		Ratio
I diploid	II triploid	III diploid	IV triploid	IV/III
9.23 $\pm$ 0.32 (17)	7 (1)	3.21	2.81	0.88
16.27 $\pm$ 0.64 (49)	13.10 $\pm$ 1.37 (10)	4.06	3.71	0.91
27.52 $\pm$ 1.40 (29)	20.56 $\pm$ 2.17 (9)	4.78	4.36	0.91
32.46 $\pm$ 1.55 (35)	24.56 $\pm$ 1.73 (16)	5.02	4.62	0.92
44.88 $\pm$ 1.24 (82)	32.88 $\pm$ 1.05 (40)	5.50	5.04	0.92
55.23 $\pm$ 1.63 (43)	36.38 $\pm$ 4.50 (8)	5.79	5.19	0.90
63.54 $\pm$ 1.52 (84)	43.87 $\pm$ 2.98 (37)	5.99	5.46	0.91
76.19 $\pm$ 1.98 (72)	53.83 $\pm$ 3.55 (12)	6.25	5.75	0.92
83.82 $\pm$ 1.72 (86)	56.71 $\pm$ 3.60 (14)	6.39	5.83	0.91
97.05 $\pm$ 2.27 (37)	63.42 $\pm$ 2.78 (26)	6.60	5.99	0.91
109.67 $\pm$ 5.22 (21)	71.33 $\pm$ 11.85 (3)	6.78	6.16	0.90
131.37 $\pm$ 3.41 (63)	80.27 $\pm$ 3.51 (22)	7.04	6.33	0.90
147.17 $\pm$ 2.76 (97)	95.26 $\pm$ 4.48 (27)	7.20	6.57	0.91
167.32 $\pm$ 3.46 (37)	100.50 $\pm$ 6.77 (8)	7.39	6.65	0.90
186.15 $\pm$ 12.89 (13)	102.39 $\pm$ 9.94 (7)	7.54	6.68	0.89

III = log I/log 2; IV = log II/log 2.

the frequency of triploids is less pronounced than in the strain we used. This assumption is supported by two lines of evidence. These are (1) high incidence of chromosome abnormalities, including triploidy, in rabbit blastocysts obtained through superovulation by PMS¹⁹, and (2) the increased frequency of triploidy and other types of chromosome anomalies observed in human spontaneous abortuses after ovulation-inducing therapy by HMG and HCG, clomiphene citrate and HCG, or HCG alone²⁰.

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Selective inhibition of neuronal GABA uptake by *cis*-1,3-aminocyclohexane carboxylic acid

It is now generally accepted that GABA is an important inhibitory synaptic transmitter in the vertebrate central nervous system. However, there remain many problems in elucidating the precise roles of GABA in the brain. One important problem is the existence of a glial pool of GABA which makes it difficult to determine the origin of GABA released during experiments designed to demonstrate the release of GABA after nervous stimulation. It was thought at one time that this problem might be overcome by utilising the uptake processes for GABA to label the neuronal GABA pools, with for example, ³H-GABA. However, it is now clear that in many areas of the brain, GABA is also taken up by glial cells and in some areas, this glial uptake may actually predominate. The transport processes for GABA in neurones and glia have remarkably similar properties but do appear to have slightly different structural requirements.

In the present study, we have found that a conformationally restricted structural analogue of GABA, *cis*-1,3-aminocyclohexane carboxylic acid (ACHC) (Fig. 1), which has previously been reported to competitively inhibit GABA transport in slices of cerebral cortex¹, selectively inhibits neuronal transport of GABA and has little or no effect on transport of GABA into glial cells. This selective inhibition was revealed by studying the uptake of ³H-GABA by isolated slices of rat cerebral cortex, frog retinae, rat retinae and rat sympathetic ganglia. Autoradiographic studies with ³H-GABA have shown a predominantly neuronal uptake in

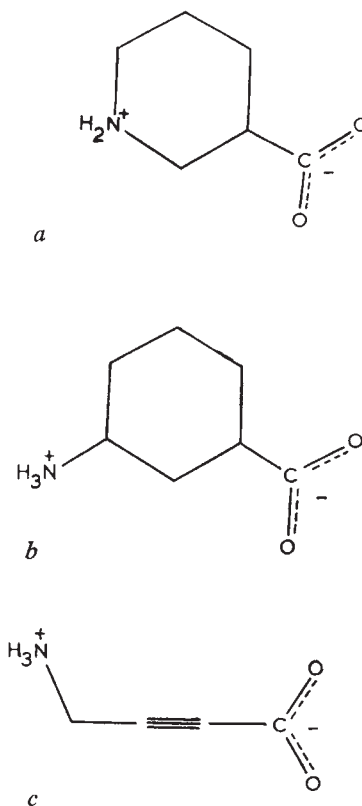


Fig. 1 Chemical line diagrams for nipecotic acid ((±)-piperidine-3-carboxylic acid) (a), (±)-*cis*-3-aminocyclohexane carboxylic acid (b), 4-aminotetrolac acid (4-aminobut-2-ynoic acid) (c).

cortical slices² and frog retinae³ and a glial uptake in rat retinae⁴ and ganglia⁵.

The effects of ACHC on ³H-GABA uptake by these tissues are summarised in Table 1. It can be seen that the uptake of ³H-GABA (0.1 μM) by cortical slices and frog retinae was 50% inhibited (IC₅₀) by ACHC at a concentration of 62 μM and 960 μM respectively but uptake into rat retinae and ganglia was unaffected by ACHC at a concentration of 1 mM. Since this concentration of ACHC was approximately 100 times the apparent *K_m* of the high-affinity GABA transport processes, these results indicate that ACHC has no significant inhibitory effect on GABA transport in glia of the rat retina and ganglion.

To study further the selectivity of ACHC on neuronal and glial systems, its ability to stimulate ³H-GABA release was investigated. In these experiments the tissue was pre-loaded with ³H-GABA and then superfused in a small chamber with medium containing aminooxyacetic acid (0.1 mM) to prevent catabolism of the accumulated ³H-GABA. During the superfusion the tissue was exposed for 4- or 6-min periods to medium containing ACHC or non-radioactive GABA. ACHC caused an increase in the efflux of ³H-GABA from superfused cortical slices and frog retinae (Fig. 2). This effect of ACHC was concentration dependent. Thus, in both tissues ACHC (10 μM) produced an approximately twofold increase in the efflux rate coefficient and this was increased further to about fivefold at a concentration of ACHC of 100 μM (cortex 5.2 ± 0.21, frog retina 4.5 ± 0.16, mean ± s.e.m. of six experiments). At 1 mM ACHC, much larger effects were seen, the rate coefficients increasing more than 14-fold (cortex 14.9 ± 1.21, retina 14.1 ± 2.01, mean ± s.e.m. 3–6 experiments). In contrast to the above tissues in which neuronal uptake of GABA predominates, the efflux of ³H-GABA from glial cells of the rat retina and ganglion was virtually unaffected by ACHC at concentrations up to 1 mM although GABA

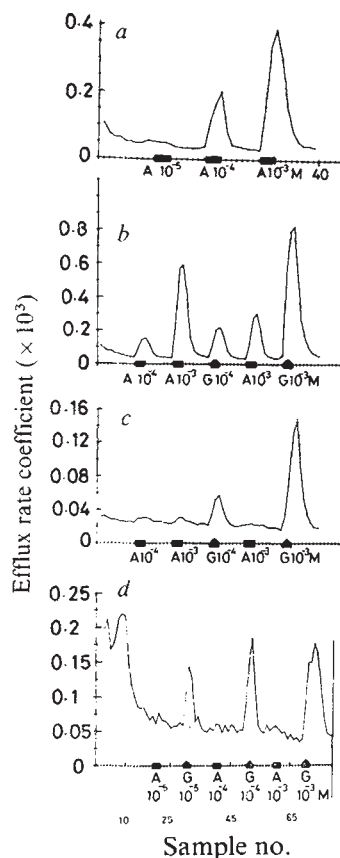


Fig. 2 Effect of GABA (G) and ACHC (A) on the rate of ³H-GABA efflux from cerebral cortex slices (a), frog retina (b), rat retina (c), and rat sympathetic ganglion (d). Tissues were given a preliminary incubation for 10 min in 5 ml Krebs' bicarbonate solution at 25 °C. ³H-GABA was then added (final concentration 1 μM) and the incubation continued for 30 min (ganglia 2 h). The tissue was then transferred to a superfusion chamber (volume 1.0 ml). Four slices of cortex (0.25 mm thick) or two retinæ were placed in each chamber and superfused at 25 °C with oxygenated medium at a rate of 1 ml min⁻¹. Fractions (2-ml) were collected and the radioactivity determined by liquid scintillation counting. The efflux of ³H-GABA from sympathetic ganglia was measured as described previously¹⁴; ganglia were superfused with medium at 0.5 ml min⁻¹, and the radioactivity in 2-min samples determined. The results are expressed as the fractional rate coefficient defined as the amount of radioactivity collected during a 2-min period divided by the arithmetic mean of the total radioactivity present in the tissue at the beginning and end of the same collection period. All media contained the GABA transaminase inhibitor, amino-oxyacetic acid, 100 μM (ganglia 10 μM), as previous experiments have shown that in the presence of this inhibitor the radioactivity accumulated and released by these tissues corresponds to > 95% ³H-GABA (refs 14 and 20–22).

'recapturing' a large proportion of the spontaneously released ³H-GABA. The alternative possibility that the stimulated release of ³H-GABA is due to a coupled exchange process⁶ with the ACHC or exogenous GABA is unlikely since in the absence of sodium ions GABA uptake is prevented and this would be expected to reduce the spontaneous GABA efflux rather than cause the marked increase that was found in the present experiments.

The above experiments indicate that (in the tissues studied) ACHC is a selective inhibitor of neuronal GABA transport and is capable of producing a net increase in the efflux of GABA from neuronal but not glial GABA pools. The properties of ACHC which confer this specificity are of interest. In aqueous solution ACHC is likely to exist as the zwitterionic form and from *pK* measurements it has been concluded that the molecule exists predominantly in the diequatorial conformation⁷. In this conformation the compound represents a partially restricted, partially folded analogue of GABA where the separation of zwitterionic centres (mean of the two N...O distances) is approximately 5.6 Å. The corresponding distance observed in the crystal structure of β-alanine is approximately 3.8 Å (ref. 8). Since β-alanine seems mainly to inhibit glial uptake of GABA⁹, the selectivity of inhibitors of GABA uptake is probably related to the separation of their zwitterionic

(1 mM) itself produced large increases in the efflux of ³H-GABA from all four tissues (Fig. 2).

The stimulated efflux of ³H-GABA produced by ACHC and by GABA itself was dependent on the presence of Na⁺ ions in the medium. In the absence of Na⁺, the spontaneous release from the tissue was increased sixfold but the addition of GABA (1 mM) or ACHC (1 mM) did not cause any additional release of ³H-GABA (Fig. 3). These effects were not due to damage of the tissue because when the Na⁺ level was restored, a stimulated efflux of ³H-GABA was obtained with both GABA and ACHC (Fig. 3). These results suggest that the increased efflux of ³H-GABA produced by ACHC (and GABA) is due to inhibition of the GABA transport process which is presumably

Table 1 Effect of ACHC and other analogues on GABA uptake

Analogue	IC ₅₀ (Concentration of compound that inhibits uptake of GABA 0.1 μM by 50%)			
	'Neuronal uptake'		'Glial uptake'	
	Cortical slices	Frog retina	Rat retina	Sympathetic ganglia
<i>cis</i> -1,3-aminocyclohexane			No effect	No effect
carboxylic acid	62 μM	960 μM	at 1 mM	at 1 mM
4-Aminotetrollic acid	330 μM	Not tested	No effect	No effect
			at 1 mM	at 1 mM (ref. 15)
Nipecotic acid	12 μM	1 mM	640 μM	200 μM
2,4-Diaminobutyric acid	66 μM (ref. 16)	Not tested	58 μM (ref. 17)	No effect
β-alanine	21 mM (ref. 9)	Not tested	390 μM (ref. 18)	at 1 mM (ref. 10)
				59 μM (ref. 14)

Slices (0.1 × 0.1 × 0.2 mm) of cerebral cortex (10 mg)¹⁹ or individual retinæ (10 mg)¹⁷ were preincubated with analogue for 10 min at 25 °C in 5 ml Krebs' bicarbonate medium. ³H-GABA was then added to give a final concentration of 0.1 μM and the incubation continued for 10 min. Desheathed ganglia¹⁰ were incubated in Krebs' solution at 25 °C for 30 min with ³H-GABA (0.1 μM) in the absence or presence of analogue. The tissue was then recovered, washed with fresh medium at 25 °C, and transferred to counting vials. The radioactivity was extracted by dissolving the tissue in Soluene-350 (Packard) and measured by liquid scintillation counting. The counts were corrected for efficiency by the channel ratio method. The inhibition of ³H-GABA uptake produced by 3 or 4 concentrations of each analogue was measured and used to obtain the IC₅₀ value¹⁸. The value at each concentration of analogue was the mean of 3–6 results and the s.e.m.s were less than 10%.

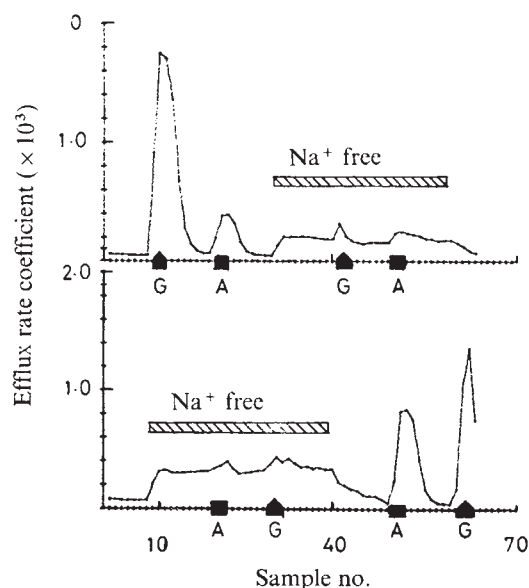


Fig. 3 Effect of Na^+ ions on the stimulated release of ^3H -GABA from cortical slices produced by ACHC (1 mM) and GABA (1 mM). Experimental details were as described in Fig. 2 except that a Tris-HCl buffered medium was used and where indicated the Na^+ in the medium was replaced with sucrose. The top panel shows that the stimulated release of ^3H -GABA produced by GABA (G) and ACHC (A) was abolished by exposure of the tissue to Na^+ free medium (indicated by the solid bar). This lack of response was not due to tissue damage because the stimulated release of ^3H -GABA was restored when the Na^+ -free medium was replaced with normal medium (bottom panel). Note also the increased spontaneous efflux of ^3H -GABA in the absence of Na^+ ions.

centres. The upper limit of this separation for interaction at glial uptake sites is apparently lower than at neuronal uptake sites. This is supported by the fact that 4-aminotetrollic acid (4ATA), where the zwitterionic centres have a minimum separation of 5.5 Å was also found to be a selective neuronal inhibitor of GABA uptake (Table 1). In contrast, nipecotic acid, which like ACHC, also adopts the chair conformation in both the solid state and aqueous solution but has a smaller separation of charged centres of approximately 4.5 Å, interacts with both neuronal and glial transport sites for GABA (Table 1).

2,4-Diaminobutyric acid (DABA) has previously been suggested as a selective inhibitor of neuronal GABA transport because it inhibits uptake by cortical slices but not by sensory⁹ and sympathetic ganglia¹⁰. However, DABA is almost equally potent in inhibiting GABA uptake in rat retina and cortical slices. This lack of selectivity is presumably due to the greater flexibility of this molecule.

In addition to its affinity for neuronal GABA transport sites, ACHC has been shown to have an inhibitory effect on the firing of central neurones. This effect is presumably mediated by GABA receptors since it is blocked by the GABA antagonist, bicuculline¹¹ (M. A. Simmonds and F. Andres-Trelles, personal communication). In contrast, we found that ACHC (3 mM) had no effect on ganglionic GABA receptors since it failed to produce the neuronal depolarising response characteristic of GABA itself¹² (Fig. 4). Nipecotic acid (3 mM) also had no effect on ganglionic neurones but depolarising responses were produced by relatively high concentrations of DABA (3 mM) and β -aminobutyric acid (BABA) (3 mM) (Fig. 4).

When the ganglionic glial cells were allowed to accumulate GABA by superfusion of the ganglion with 1 mM GABA for 1 h (ref. 14), it was found that the depolarisations produced by DABA and BABA were potentiated and

nipecotic acid, which was previously without effect, now produced a prolonged depolarisation. In contrast, ACHC still did not depolarise the ganglionic neurones (Fig. 4). The potentiation of the depolarising responses of DABA and BABA after loading of the glial cells with GABA is due to their affinity for the glial transport sites. These compounds inhibit the reuptake of GABA and so increase the net release of GABA from the glial cells as illustrated previously (Fig. 2). This indirect activation of ganglionic GABA receptors is even more strikingly demonstrated in the case of nipecotic acid which produced no depolarisation at all until after the glial cells were loaded with GABA but then produced a prolonged depolarisation of the ganglionic neurones (Fig. 4). Unlike nipecotic acid, ACHC did not depolarise ganglionic neurones after the glia were loaded with GABA. This failure to show an indirect effect on ganglionic GABA receptors is consistent with this compound's lack of affinity for glial uptake sites. However, since

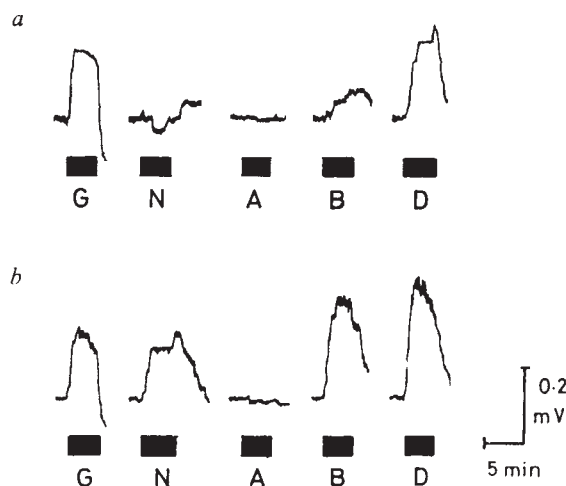


Fig. 4 Depolarising responses of an isolated superior cervical ganglion to GABA (G, 3 μM), ACHC (A, 3 mM), β -aminobutyric acid (B, 3 mM), 2,4-diaminobutyric acid (D, 3 mM) and nipecotic acid (N, 3 mM). The ganglion was obtained from a Wistar rat under urethane anaesthesia and desheathed before superfusion with Krebs' solution (1 ml min⁻¹) at 25 °C. Two non-polarisable Ag^+/AgCl electrodes were placed in contact with the postganglionic trunk and ganglion body and connected across a Servoscribe 1-s potentiometric recorder¹³. Responses correspond to an increase in the potential difference measured across these electrodes. Responses were obtained before (a) and after (b) superfusion of the ganglion with 1 mM GABA for 60 min followed by a further 60 min superfusion with Krebs' solution¹⁴. Each drug was in contact with the tissue for 4 min, indicated by the solid bars below the record, and was applied at intervals of 15–20 min (gaps between records). Aminooxyacetic acid (10 μM) was present in all solutions. Note that in b responses to nipecotic acid, β -aminobutyric acid and 2,4-diaminobutyric acid were larger than in a, whereas ACHC still failed to evoke a response.

ACHC does have affinity for neuronal GABA transport sites, this raises the interesting possibility that the inhibitory effects of this compound (and perhaps nipecotic acid) on the firing of central neurones is mediated indirectly by increasing the net efflux of endogenous GABA from neuronal pools. An alternative possibility which cannot be excluded at present is that the receptors for GABA on ganglia and on central neurones have different specificity requirements.

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Distribution of ions in a fluid-transporting epithelium determined by electron-probe X-ray microanalysis

STUDIES of fluid transport in epithelia have in general been limited to measurements of the composition of the transported fluid and the electrochemical gradients against which transport occurs¹⁻³. We have now used electron-probe X-ray microanalysis to measure the intracellular ionic concentrations. The data show that Na, K and Cl are not uniformly distributed within the cells; that the basal lamina is not entirely a passive membrane open to small ions, and that, in the particular epithelium studied, the stimulation of secretion greatly increases the intracellular Na concentration. In addition, the results do not support the standing gradient theory of fluid secretion.

Our technique (see legend to Fig. 1) is a refinement of that reported earlier⁴: tissue in known physiological condition is quench-frozen at -180°C , sectioned and transferred to the cold stage of an X-ray microanalyser. By the maintenance of low temperature, the water in the tissue is retained as ice and the distribution of soluble constituents is preserved—as has now been confirmed by experiments on several animal tissues (ref. 5, and our unpublished work). No chemical fixation or dehydration is used.

We studied the fluid-secreting upper portion of Malpighian tubules from the blood-sucking insect *Rhodnius prolixus*. Depending on the composition of the bathing medium, this epithelium secretes fluid rich in either Na or K or both⁶. Furthermore, the normally low rate of fluid secretion can be increased (up to 1,000 times) by treatment with diuretic hormone from the insect, or with 5-hydroxytryptamine (5-HT)^{7,8}.

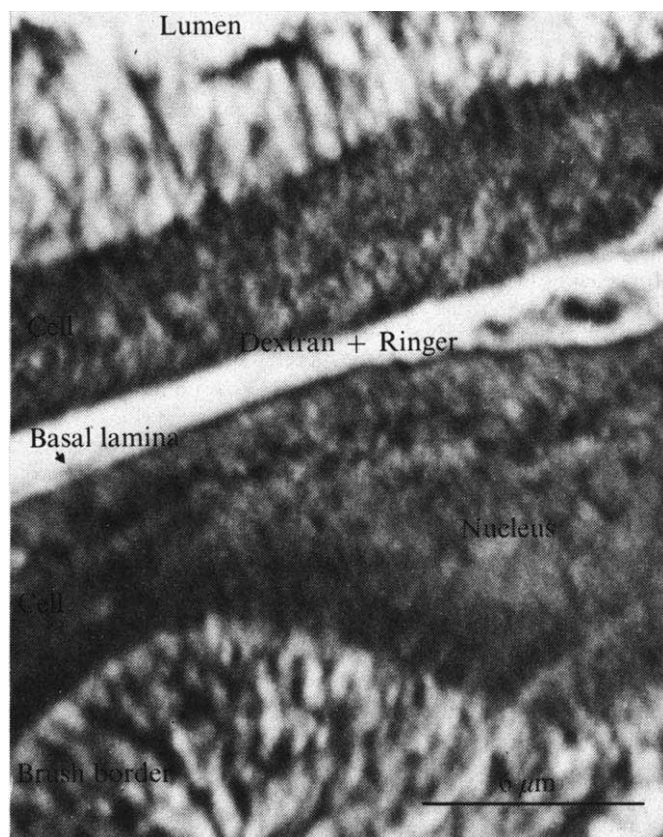


Fig. 1 Scanning transmission electron micrograph of a deep-frozen hydrated section of the upper secretory portion of a Malpighian tubule from *Rhodnius prolixus*, showing two parts of a tubule, side by side, with various tissue components as labelled. Because of the wide range of contrast in the image, the basal lamina is not well shown in this micrograph. Tubules from unfed, fifth-instar *Rhodnius* were dissected in saline containing (mM) K^+ 20, Na^+ 132, Cl^- 160, Ca^{2+} 2, Mg^{2+} 9, H_2PO_4^- 4, HCO_3^- 10, glucose 34, together with 10–20% (w/v) dextran (molecular weight about 237,000). The dextran was included to reduce ice-crystal damage during quenching and to improve sectioning at low temperature; physiological experiments showed that it had no significant effect on secretion rates. For stimulated tubules, about 10^{-5} M 5-HT was added. (Other tubules from the same insect were isolated in identical saline containing ^{22}Na or ^{36}Cl , so that the concentrations of these ions in the secreted fluid could be measured for comparison with the microprobe results.) Lengths of tubules were mounted in a drop of dextran-saline on either silver or copper pins from a modified Cryokit attachment for the LKB Ultratome III (B. L. G. and N. G. F. Cooper, in preparation) or on small chucks for a cryomicrotome (SLEE Instrument Co.)⁴. They were then quench frozen at -180°C by plunging into Freon-13 (monochlorotrifluoromethane) maintained as a solid-liquid slush with liquid nitrogen, and transferred to the microtome. Sections (1–2 μm thick) were cut with steel knives at -80°C in the SLEE microtome, or with glass knives at -120° to -140°C in the LKB Cryokit. The sections were picked up on aluminium alloy collars covered with aluminised nylon films and loaded on to the low-temperature stage of a JEOL JXA-50A X-ray microanalyser. Special transfer devices were used to maintain the sections below -150°C to protect them from dehydration. Throughout the observations the specimen stage was kept at -170°C ; in these conditions the loss of mass from areas under the beam is unlikely to be significant^{4,20}. After each set of microanalyses the state of hydration of the section was checked as follows: the specimen holder was removed from the cold stage and held at the end of the loading rod within the column under high vacuum for 1–2 min. During this time the section warmed up and dried rapidly. The holder was then returned to the cold stage and analysis was repeated. From the change in X-ray continuum after drying, without parallel changes in either the characteristic counts or the distribution of elements, the section illustrated here and the other sections used for the results in Figs 2 and 3 were estimated to have retained 90–80% of their original water contents during the first analysis.

Figure 1 is a scanning transmission electron micrograph of a 1- μm thick, deep-frozen, hydrated section of a *Rhodnius* Malpighian tubule stimulated with 5-HT and prepared as described in the legend. To analyse the distribution of elements, a focused electron beam about 100 nm in diameter was localised in different areas at magnifications of 5,000–20,000. Characteristic X-ray signals from the $K\alpha$ radiations of Na and K were recorded from diffracting spectrometers while a complete X-ray energy spectrum was obtained simultaneously from a Kevex Si(Li) detector. The energy spectrum provided data on K, Cl and other elements and on the continuum or "white" radiation used as a measure of local mass in the specimen⁹. After correction for background and for contributions to the mass counts from the substrate film and the specimen holder, each measurement was converted into an elemental mass fraction by comparison with the corresponding counts for known concentrations in dextran-saline in the same section^{9,10}. These values are given as mmol per kg wet weight of the areas indicated in Figs 2 and 3, which summarise the results of several experiments with both unstimulated and 5-HT-stimulated tubules. The salient results are as follows.

The fluid in the lumen of unstimulated tubules was different from the fluid secreted at a high rate by stimulated tubules. The former was much richer in potassium, much

poorer in sodium, and poorer in chlorine. It also contained a high concentration of phosphorus, presumably as inorganic phosphate.

In the cytosol the distribution of ions was non-uniform, but with respect to the main cell cytoplasm (areas 5 and 6 in Fig. 2), the only significant consequence of stimulation was an increase of about 30 mM in intracellular Na.

In the region of the brush border, our first analysis (areas 9–11 in Fig. 2) did not distinguish between the microvilli and the adjacent extracellular spaces; these data do not show a systematic variation in Na, K or Cl with distance into the lumen in the unstimulated tubules, but in the stimulated tubules the levels of all three ions increase towards the lumen. With more careful localisation of the probe (Fig. 3) we found significant differences between the microvilli and the extracellular spaces, and gradients along the length of both. In stimulated tubules, the increase of Na and K towards the lumen was particularly marked, so that both elements seemed to be significantly more concentrated towards the luminal end of the extracellular space than in the main lumen.

The basal lamina contained a higher concentration of K and lower concentrations of Na and Cl than the dextran-saline (Fig. 2, area 2). Phosphorus concentrations in the lamina were higher than elsewhere.

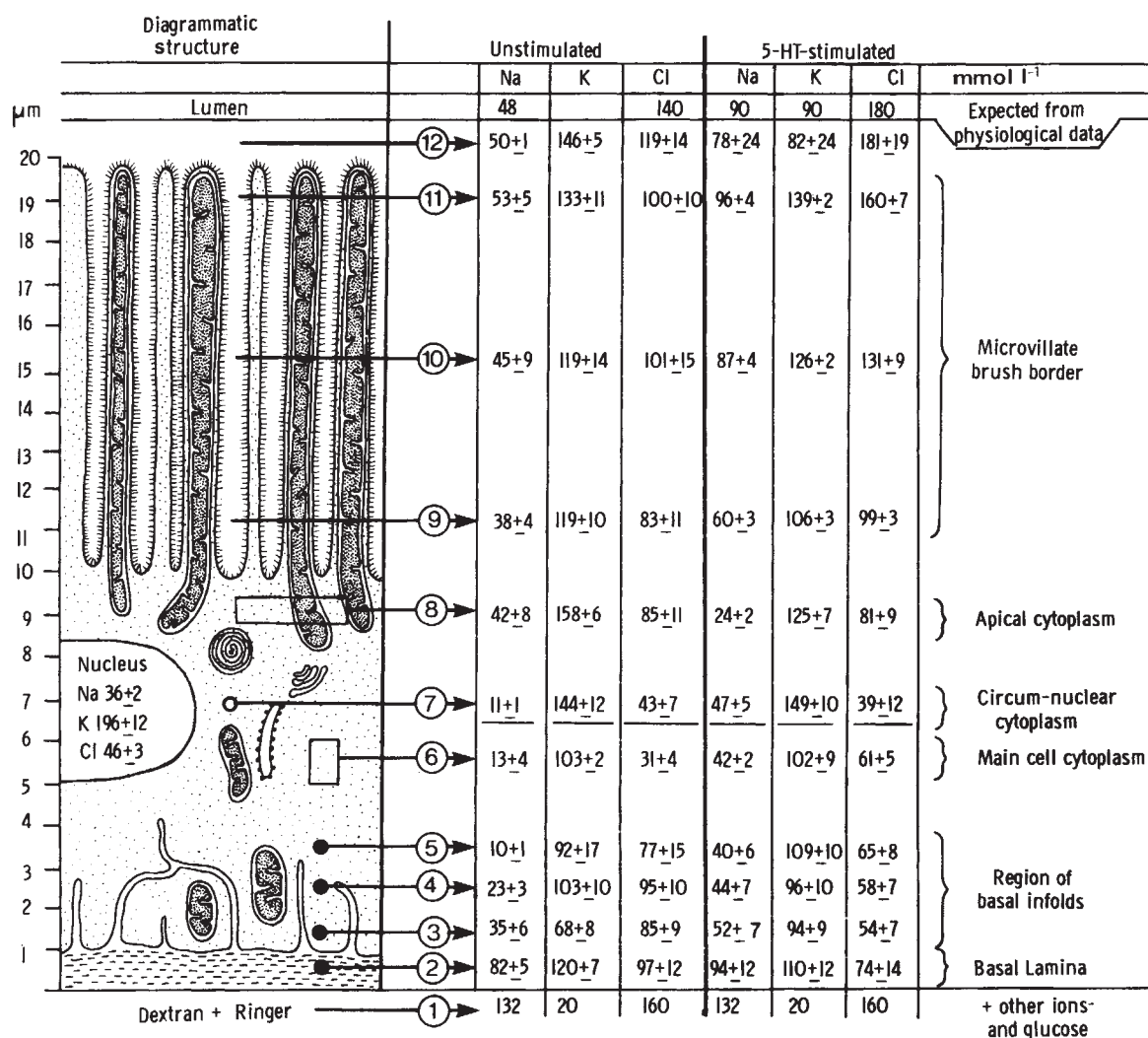


Fig. 2 Summary of microanalytical results obtained from stimulated and unstimulated Malpighian tubules of *Rhodnius*. The diagrammatic representation of the structure was prepared from conventional electron micrographs, and shows positions (but not actual sizes) of areas scanned by the probe. Measured concentrations of Na, K and Cl are expressed in mM (± 1 s.e.) per kg wet weight. The contents of the lumen in the unstimulated tubules also contained 20–30 mmol of phosphorus.

Our measurements on the fluid in the lumen confirm the validity of the technique. For stimulated tubules, the results for Na, K and Cl are in reasonable agreement with the findings of earlier physiological studies⁶. The fluid secreted by unstimulated *Rhodnius* tubules had not been analysed previously, but parallel isotopic measurements gave values of 50 mM for Na and 140 mM for Cl, again in good agreement. The unstimulated secretion is remarkably similar in composition to the product of the relatively slowly secreting Malpighian tubules of other insects, such as *Calliphora*^{8,11}.

The discovery of increased Na levels within the stimulated secretory cells should be considered together with the concurrent elevation of Na in the lumen. The finding is in accord with parallel measurements with ²²Na and ⁴²K, which show that in stimulated tubules the ionic composition of the secreted fluid is closely dependent on that within the cells (S.H.P.M., J. L. Wood and W. R. Harvey, in preparation). To further interpret the data we must take into account a special feature of the *Rhodnius* system: whereas the rate of fluid secretion by most insect Malpighian tubules depends strongly on the concentration of potassium ions in the bathing medium⁸, stimulated *Rhodnius* tubules can maintain high rates of secretion of Na-rich fluid in K-free media. All the available data seem to suggest that in *Rhodnius*, 5-HT (and presumably diuretic hormone *in vivo*) induces rapid entry of sodium ions into the tubule cells, and the elevation of intracellular Na is essential to a rapid secretion of sodium-rich fluid. (Similar mechanisms have been postulated for the action of hormones and neurotransmitters in several vertebrate epithelia¹²⁻¹⁶, although the

intracellular ionic changes have not been demonstrated conclusively in these tissues¹⁷.) One may postulate further that secretion is driven by an apical pump which transports Na and K in proportion to their availability.

In the brush border region, our measurements do not distinguish cleanly between microvilli and the channels between them, because the spread of a 100-nm probe in a section 1 μ m thick is too large compared with the transverse dimensions of the microvilli and the channels. The presence of mitochondria in the microvilli also adds to the scatter in the results. But the observed distributions should be qualitatively valid, and they bear directly on the way in which the movement of water may be coupled to an active movement of ions to maintain near isotonicity of the transported fluids in various animal epithelia. Among several models^{5,12}, the "standing gradient osmotic flow" hypothesis¹⁸ has been applied widely to both vertebrate and invertebrate tissues², but support¹⁸ and criticism^{19,20} of this model have been based on theory alone, in the absence of direct measurements of the gradients. In Malpighian tubules it has been suggested that the cytoplasmic processes in the region of the basal infolds and/or the extracellular spaces between the microvilli in the brush border constitute the forwardly-directed channels in which standing gradients are generated⁸. Our observations show that in *Rhodnius*, transport across the tubule brush border is unlikely to be explained in this way since the direction of the observed gradients is the reverse of that required by the model.

The sum of the Na and K levels in the brush border region is well above the Cl level. This "anion deficit" may

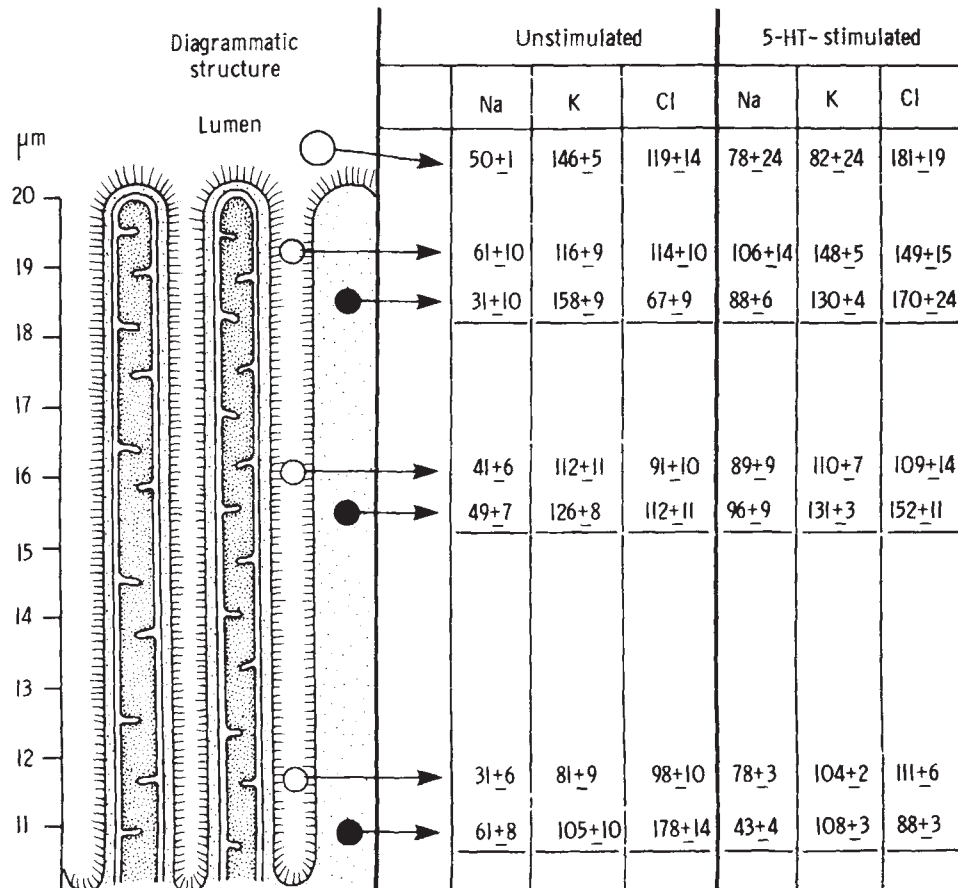


Fig. 3 As Fig. 2, but showing detailed distribution of Na, K and Cl in the microvillar region. The mitochondrion has been omitted from the marked microvilli, for clarity.

be taken as evidence of ion binding to extracellular organic material, possibly the anionic glycocalyx demonstrated by electron microscopy in other tissues^{21,22}.

In the brush border region, we need microanalyses with better spatial resolution, to be obtained with thinner sections of tissue, and we need information about the proportion of ions bound and the amount of free water present. Further experiments are in progress.

In the basal lamina, the high concentrations of potassium have now been found in other insect tissues as well (our unpublished work). The observation cannot be attributed to the limited analytical spatial resolution, which can only tend to obscure existing sharp peaks in concentration. The basal lamina has generally been regarded to be insignificant in relation to fluid transport, as experiments have shown it to be freely permeable to uncharged macromolecules²³ and colloidal particles²⁴. But our results suggest a preferential binding of K by the basal lamina, possibly to anionic sites on collagen or acidic proteoglycans²⁵. The presence of phosphoglycans is suggested by the high concentration of phosphorus, and of anionic sites by the fact that the basal lamina selectively binds lanthanum (unpublished work of B. L. G.). The implication for fluid transport is that the basal lamina, although apparently permeable to quite large neutral molecules, may nevertheless preferentially restrict the movement of some ions. Particularly in the presence of the large net fluxes of ions and water, observed in stimulated Malpighian tubules, the result may be a significant difference in composition between the external medium and that bathing the cells.

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Comparison of the biological activities of an insect and a crustacean neurohormone that are structurally similar

THE molecular structure of locust adipokinetic hormone (AKH) (ref. 1), closely resembles that of the only other arthropod neurohormone to be fully characterised, prawn red-pigment-concentrating hormone (RPCH) (ref. 2). The first eight residues of the decapeptide, AKH, are homologous with the octapeptide, RPCH, except for threonine instead of serine as residue 5, and asparagine instead of glycine as residue 7, as shown below.

RPCH: PCA-Leu-Asn-Phe-Ser-Pro-Gly-Trp-NH₂

1 2 3 4 5 6 7 8

AKH: PCA-Leu-Asn-Phe-Thr-Pro-Asn-Trp-Gly-Thr-NH₂

1 2 3 4 5 6 7 8 9 10

Although both hormones are the products of neuroendocrine organs, such close similarities in structure were somewhat unexpected, as the two hormones have completely different physiological actions. AKH in the locust controls lipid utilisation for energy during prolonged flights, by stimulating the release of diglycerides from the fat body³, and regulating their utilisation by the flight muscle⁴. RPCH in the prawn stimulates the concentration of pigment within the red chromatophores⁵. We present here evidence that the two hormones can reproduce each other's effects when cross tested on members of the two arthropod groups.

Earlier work^{6,7} had demonstrated that extracts of corpora cardiaca from several insects were able to concentrate the red chromatophore pigments in prawns and crayfish. In this preliminary study we have investigated the effects on pigment

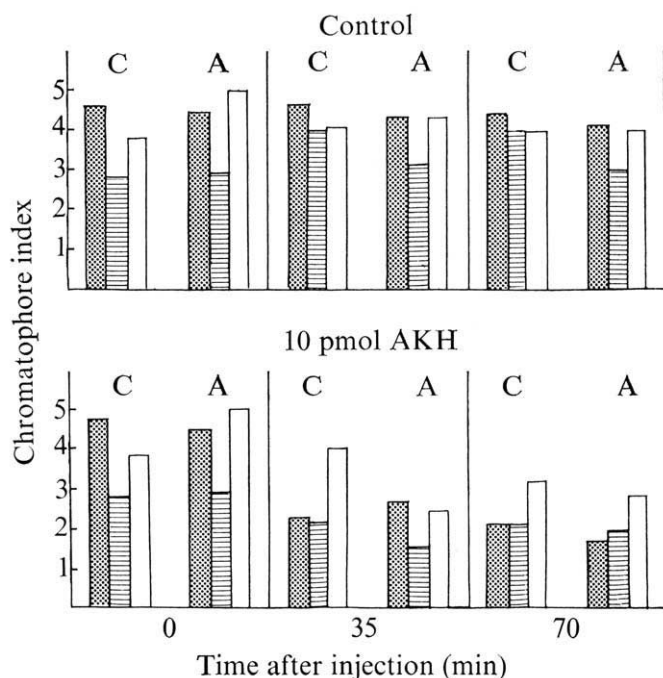


Fig. 1 Effects of AKH on the chromatophores of *Palaemon squilla*. The prawns were kept in seawater on a black background. Samples of 10 pmol AKH in 10 μ l 2.7% NaCl were injected into six animals; five control animals were not injected but were kept in identical conditions. The average chromatophore indices from groups of 20 chromatophores of each type on the carapace (C), and on the margin of the fifth abdominal sclerite (A) were estimated for each animal, and the results from each animal averaged. Stippled columns, red chromatophores; hatched columns, black chromatophores; open columns, yellow and white chromatophores.

Table 1 Effects of *Dichelopandalus* eyestalk extract and AKH on red chromatophores of *Dichelopandalus bonnieri*

Sample injected	No. of animals	Chromatophore index at various times after injection			
		0 min	20 min	45 min	100 min
Saline	4	4.8	4.9	4.8	4.6
Extract of 0.5 <i>Dichelopandalus</i> eyestalk	5	4.8	1.8		2.8
AKH : 2.0 (pmol)	4	4.8	2.8	1.7	3.8
1.0	4	4.8	Slight effect		
0.2	4	4.9	No effect		

Intact *Dichelopandalus bonnieri* kept in seawater on a red background were injected with 25 μ l 2.7% NaCl in which the test samples were dissolved. Each prawn was approximately 4 g wet weight. The chromatophore indices⁸ for groups of 20 red chromatophores on the carapace and abdomen of each animal were estimated, and the values for each animal averaged. Extracts were prepared, immediately before injection, by disrupting the eyestalks in 2.7% NaCl.

concentration of injecting pure AKH (ref. 1) into different crustacean species. We have also examined the ability of prawn eyestalk extracts to mobilise lipids in locusts. The limited supply of prawns available to us necessitated the use of extracts of eyestalks, rather than pure RPCH.

Table 1 shows the effects on red pigment concentration of injecting both a saline extract of *Dichelopandalus bonnieri* eyestalks, and AKH, into intact *Dichelopandalus*. The prawns were kept on a red background throughout the experiment, so that before injection the red pigment in the chromatophores was fully dispersed. The eyestalk extract and AKH both concentrate the red pigment markedly. Similar blanching effects were observed with methanolic extracts of *Dichelopandalus* eyestalks. When injected with 10 pmol AKH the prawns blanched completely within 5 min, whereas with 2 pmol the maximum effect was observed after 45 min. 1 pmol AKH is required to achieve some blanching in intact *Dichelopandalus*. *Pandalus montagui* also blanched slightly with this level of hormone, though neither species responded to 0.2 pmol AKH. In *Dichelopandalus* with ligatured eyestalks, and thus with the red pigment fully dispersed, three out of five animals blanched slightly with 0.2 pmol AKH; all five animals showed marked blanching 10 min after the injection of 1 pmol AKH. Hence the 'threshold' dose of AKH necessary to achieve an effect in these prawns is between 0.2 and 1 pmol.

The effects on pigment concentration of injecting AKH into

dark-adapted *Palaemon squilla* are illustrated in Fig. 1. Pigment dispersion in the red, black, yellow and white chromatophores of both the carapace and abdomen was measured. 10 pmol AKH concentrated the red and black pigments markedly and had a lesser effect on the yellow and white pigments. The effect was greatest 1 h after injection; after 2.5 h the pigments were fully dispersed again. These results may be compared with earlier studies⁹ on the effects of pure RPCH from *Pandalus borealis* on *Palaemon adspersus* chromatophores. 0.05 pmol RPCH concentrated both red and white pigments though the maximum white chromatophore response was smaller (chromatophore index (CI) 2.5) than the red chromatophore response (CI 1.0).

Dark-adapted *Crangon crangon* injected with 10 pmol AKH became very pale within 5 min of injection. The chromatophore indices of the red and black body chromatophores, and the red chromatophores on the telson, all decreased from CI 4–5 to CI 1.0 in this time and these effects persisted for 45 min. The white and yellow body chromatophores (CI 3.0) were unaffected during this time as were the black chromatophores on the telson (CI 4.5). The dispersions of black pigment in the body and telson are controlled by different factors¹⁰; our findings suggest that AKH is mimicking the body-concentrating factor.

The adipokinetic activities of methanolic extracts of eyestalks from *Dichelopandalus* and *Palaemon* injected into *Locusta migratoria* were measured using the procedure described earlier. The dose-response curves (Fig. 2) demonstrate that both extracts are able to mobilise lipids in locusts. The maximum response elicited by the *Dichelopandalus* extract was lower (11 units per locust) than that produced by the *Palaemon* extract (20 units). The maximum *Palaemon* response is, however, comparable with the maximum response of AKH (20 units per locust)¹.

We have shown that methanolic extracts of *Dichelopandalus*

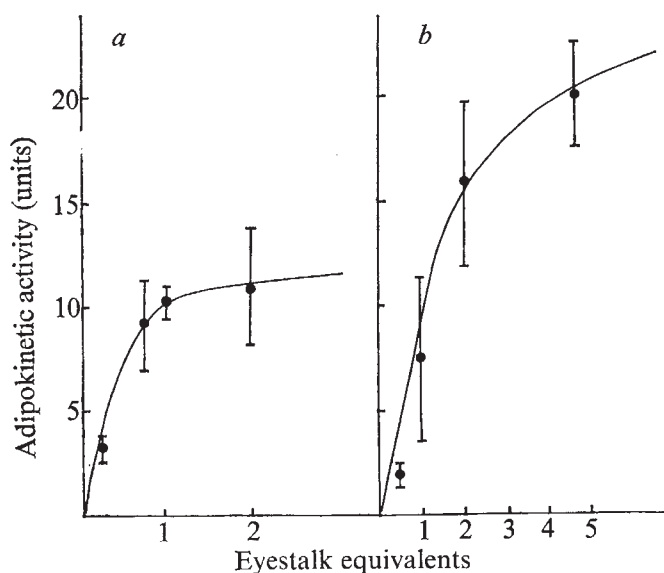


Fig. 2 Dose-response curves for the adipokinetic activity of methanolic extracts of eyestalks from *Dichelopandalus bonnieri* (a) and *Palaemon squilla* (b). The extracts, reconstituted in 20 μ l simple saline were injected into adult male *Locusta migratoria* 19–21 d old and adipokinetic activities were measured as before¹. The results represent the mean activity $\pm$ s.e. from six locusts for the *Dichelopandalus* extract and the mean activity $\pm$ s.e. from four locusts for the *Palaemon* extract.

Table 2 Effects of thermolysin digestion on the adipokinetic activity of AKH and methanolic extracts from *Dichelopandalus* and *Palaemon*

Sample injected	Adipokinetic activity (units) of sample		Comparison of means
	Undigested	Digested with thermolysin	
Simple saline	1.1 $\pm$ 0.7	2.1 $\pm$ 0.4	
5 pmol AKH	19.3 $\pm$ 2.1	2.5 $\pm$ 1.4	$P < 0.001$
Extract from 1.5 <i>Dichelopandalus</i> eyestalks	9.5 $\pm$ 0.4	2.8 $\pm$ 0.4	$P < 0.001$
Extract from 1.5 <i>Palaemon</i> eyestalks	9.1 $\pm$ 0.7	1.8 $\pm$ 0.4	$P < 0.001$

1 nmol AKH, methanolic extracts from eight *Dichelopandalus* eyestalks, and from eight *Palaemon* eyestalks, were each dissolved in 30 μ l 0.067 M NH_4HCO_3 containing 0.15 μ mol CaCl_2 and 0.1 nmol thermolysin. The solutions were incubated for 4 h at 37 $^\circ\text{C}$, heated to 100 $^\circ\text{C}$ for 5 min and NH_4HCO_3 and water removed by lyophilisation. Identical samples, with no enzyme, were treated in the same way. The residues, redissolved in simple saline were assayed for adipokinetic activity¹ in adult male *Locusta* 16–19 d old. The results represent the mean activity $\pm$ s.e. from four locusts.

eyestalks contain a factor with red pigment-concentrating activity. However, to establish whether the lipid-mobilising activity of these extracts is attributable to RPCH, we carried out further experiments. Both AKH¹ and RPCH² are cleaved by thermolysin; this cleavage of AKH destroys all its adipokinetic activity. Table 2 shows that thermolytic digestion of the eyestalk extracts destroys their adipokinetic activities. In addition, both the *Dichelopandalus* and *Palaemon* extracts elicited marked hyperglycaemic activity when injected into cockroaches (W.M. and J.V.S., unpublished) as does AKH (Jones, J. V. S., and W.M., in preparation). From these observations, we can conclude that the factor with adipokinetic activity present in the prawn eyestalk extracts is either RPCH or a peptide which closely resembles RPCH and AKH.

Thus locust AKH can stimulate pigment concentration in prawns and shrimps, and a peptide from crustacean eyestalks, probably RPCH, can mobilise lipids in locusts. As no pure RPCH was available, we were unable to estimate the relative potencies of RPCH and AKH in these two systems. Our findings suggest that these molecules are sufficiently similar in structure to fit on to the hormone receptors in either system; how good each 'fit' is may be indicated by comparing the molar activities of the peptides in each system. We hope to carry out such experiments in the near future.

Up to this point, our detailed knowledge of the comparative endocrinology of arthropods has been restricted to the control of moulting. The moulting hormone, 20-hydroxyecdysone, is of common occurrence in arthropods and acts on the same target, namely the epidermis, in different groups. Our results suggest that the evolution of neurosecretory hormone systems in arthropods is of a different pattern. It seems there has been a similar evolution of hormone structures, but an independent evolution of the target tissues. Examples of structurally similar peptide hormones acting on different targets in different classes are well documented among the vertebrates¹¹.

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Likely pre-Suez occurrence of a Red Sea fish *Aphanius dispar* in the Mediterranean

THE construction of artificial waterways connecting different faunal provinces provides biologists with an opportunity to observe ecological, biogeographic and evolutionary changes. But recognition of change requires a thorough knowledge of conditions before construction. The opening of the Suez Canal in 1857 is a case in point. More than thirty species of Red Sea fishes have been recorded as

colonising the Mediterranean since the opening of the canal¹, but because no reliable systematic ichthyological collections were made in the eastern Mediterranean before 1857, these new records must be judged with caution. We report here comparative electrophoretic evidence which suggests that the Red Sea cyprinodontid, *Aphanius dispar*, first reported along the Israeli coast in 1947 (ref. 2) and therefore considered a Suez migrant, has been a permanent Mediterranean resident for a long time.

A. dispar (Rüppell) is a small, littoral, euryhaline fish widely distributed in the Indian Ocean, which also occurs along both coasts of the Sinai Peninsula and in the Suez Canal. In the Mediterranean, it was reported at Port Said³ and subsequently along the Israeli coast from Atlit, Tel Aviv, and Caesarea^{2,4}. In addition, several subspecifically distinct populations (*A. dispar richardsoni* (Boulenger)) occur as isolates in freshwater pools along the periphery of the Dead Sea. These Dead Sea fishes have been living in isolation since the early Pleistocene⁵ and differ from conspecifics in male breeding coloration⁶. But all populations of the species have the same gross karyotype, cross freely in aquaria and are morphologically indistinguishable^{6,7}.

We collected population samples of *A. dispar* at five localities from the Mediterranean, Red Sea and Dead Sea (Fig. 1). All specimens were examined for genetic variability at nineteen putative isoenzyme loci by standard methods of starch gel electrophoresis⁸ (Table 1). Genetic similarity between all possible pairs of these populations was calculated by means of Rogers' coefficient (S_R); the values are provided in Table 2.

In general, excluding comparisons between subspecies and karyotypically differentiated populations (which could easily be regarded as sibling species), virtually all intra-specific estimates of genetic similarity (S_R) in a wide variety of organisms have been greater than 0.75^{10,11}. As expected, both population pairs within the Red Sea and Dead Sea exhibit a very high degree of similarity. The estimates of similarity for *A. dispar* between the Mediterranean, Red Sea and Dead Sea are in sharp contrast, however (Table 2). Because *A. dispar* was presumed to have entered the Mediterranean only recently through the Suez Canal^{2,12}, it was expected that this population sample would approximate the level of divergence observed among Red Sea samples. Instead, the degree of difference between Mediterranean and Red Sea samples is as large as that between either sample

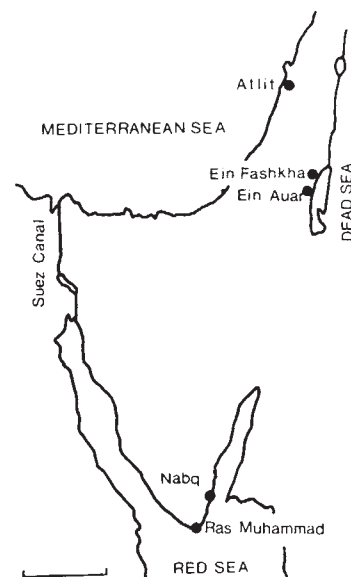


Fig. 1 Sampling localities of *Aphanius dispar* in Israel. The scale equals 100 km.

Table 1 Genetic variation at 19 loci in five populations of *A. dispar*

Sample area	Locality	Heterozygosity*	Average no. of alleles per locus	Unique alleles†
Mediterranean	Atlit	6.27	1.26	6
Red Sea	Nabq	2.39	1.37	8
Red Sea	Ras Muhammad	6.00	1.26	
Dead Sea	Ein Fashkha	5.10	1.26	6
Dead Sea	Ein el Ghuweir	4.56	1.11	

Thirty individuals were examined from each locality. With the exception of the assay for lactate dehydrogenase (LDH), all proteins were examined from whole-body homogenates diluted 1:1 with deionised water. LDH was examined from individual homogenates of eye. Electrophoretic procedures and histochemical staining were similar to those described before⁸. Specimens were examined for the following proteins (number of presumed loci in parentheses): esterase (2), LDH (3), malate dehydrogenase (2), phosphoglucumutase (3), isocitrate dehydrogenase (1), general protein (2), α -glycerophosphate dehydrogenase (1), 6-phosphoglucuronate dehydrogenase (1), phosphoglucose isomerase (2), aldolase (1), and amino peptidase (1).

*Heterozygosity was calculated as the average observed number of heterozygotes per locus, expressed ($\times 100$).

†Unique alleles are those alleles which were observed in only one out of the three sample areas.

and the Dead Sea isolates, whose last conspecific contact was during the early Pleistocene. Such genetic differences are of the order normally associated with interspecific comparisons, where long periods of isolation are indicated¹³. We conclude therefore that *Aphanius dispar* was extant in the Mediterranean before the construction of the Suez Canal.

Our conclusion reinforces speculations about the pre-Suez occurrence of *A. dispar* in the Mediterranean. This species has previously^{14,15} been considered a likely candidate for pre-Suez migration because of its wide salinity tolerance and the high probability of early interoceanic contact facilitated by ancient channelling¹⁶ and eustatic fluctuations.

A. dispar prevented survival in a cooling Mediterranean¹², but measurements of thermal tolerance (our unpublished results) and recent palaeoclimatological estimates of the Pleistocene Mediterranean²⁰ invalidate such concern. There is no support for an extinction of this population. A further argument is by analogy to the occurrence of a congeneric species, *Aphanius fasciatus*. This fish is a widely distributed Mediterranean endemic common in the littoral. After the opening of the Suez Canal, it was reported in abundance from Lake Timsah, 75 km south of the Mediterranean, yet it was formally recorded from the Israeli coast only in 1954¹⁸. Thus, if an ecologically similar and widely distributed Mediterranean resident was not detected along the Israeli

Table 2 Genetic similarity (S_R)^a among five Israeli populations of *A. dispar*

	Mediterranean Sea Atlit	Nabq	Red Sea Ras Muhammad	Ein Fashkha	Dead Sea Ein el Ghuweir
Atlit	—	0.721	0.716	0.596	0.580
Nabq	—	—	0.972	0.728	0.708
Ras Muhammad	—	—	—	0.731	0.721
Ein Fashkha	—	—	—	—	0.979
Ein el Ghuweir	—	—	—	—	—

At least two alternative explanations for our observations, consistent with the idea of recent Suez migration, merit attention. First, stochastic events associated with colonisation may have reduced the level of variability and provided a biased sample of founding Red Sea genes. Although a normal level of heterozygosity could easily become re-established if population size increased rapidly, the average number of alleles per locus would recover much more slowly from a severe bottleneck¹⁷. The Mediterranean sample, however, is not distinguished by either homozygosity or a lower average number of alleles compared with conspecifics (Table 1). More convincingly, the presence of unique alleles in all areas sampled (Table 1) can be taken as evidence of substantial isolation and absence of gene flow. Second, the source material for the Mediterranean colonisation may not have come from the Red Sea itself, but from some geographical isolate within the Suez area. For example, the founders could have come from a genetically differentiated population isolated in the Great Bitter Lakes. While this suggestion cannot be excluded, we consider it unlikely (see below).

Several further arguments support our contention that *A. dispar* is of pre-Suez occurrence in the Mediterranean. The progenitors of the Dead Sea subspecies must have colonised the Jordan drainage system from the Mediterranean^{18,19}. The idea that the extant population of *A. dispar* in the Mediterranean is of recent origin is associated with the assumption that the original founding population became extinct. It has been argued that the thermophilic nature of

coast until very recently, what degree of confidence can be placed in early negative reports for *A. dispar*?

Our findings have implications for future oceanic waterways. Clearly, only extremely comprehensive systematic surveys can provide the necessary baselines for evaluating the impact of major faunal interchange. We follow many others^{21,22}, in reiterating this concern with respect to the proposed and much debated sea level canal in Panama.

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Chromosomal recombination and mapping in *Rhizobium leguminosarum*

IGNORANCE of the genetics of the nitrogen-fixing symbiosis between *Rhizobium* species and leguminous plants is due chiefly to the lack of a good system for genetic mapping in *Rhizobium*. Although gene transfer by transformation and transduction have been reported in several *Rhizobium*

species^{1,2}, the only linkage map has come from studies of conjugation in a non-nodulating *R. lupini* strain³ and a genetic analysis of symbiotically defective mutants has not been possible. *R. leguminosarum* is particularly suitable for genetic studies since it nodulates the pea (*Pisum sativum*), a genetically well known legume^{4,5}. Initial studies with many *R. leguminosarum* wild types failed to demonstrate recombination⁶ although P group R factors could be transferred between them^{6,7}. These plasmids have wide host ranges⁸ and have been shown to mediate the transfer of chromosomal genes in *Pseudomonas*⁹, *Escherichia*¹⁰ and *Acinetobacter*¹¹. R68.45 is a derivative of the P group R factor (R68) isolated by Haas and Holloway⁹ for having much better sex factor activity in *P. aeruginosa* than R68 and other R factors. We report here that it behaves in a similar manner in nodulating strains of *R. leguminosarum* and can be used for genetic mapping in this species.

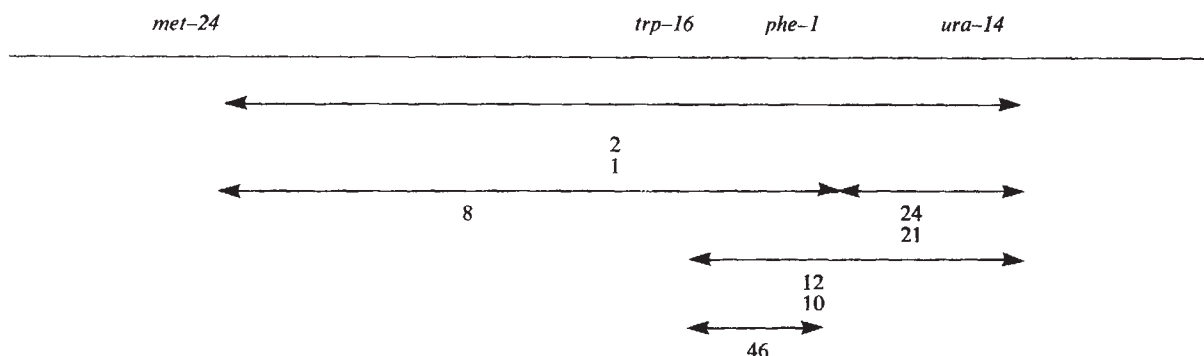
Table 1 Analysis of four-factor crosses: *phe-1 ser-1* (R68.45) × *ura-14 met-14 rif-74* (cross A) and *phe-1 ser-1* (R68.45) × *ura-14 trp-16* (cross B)

(a) Genotypes of recombinants from selective media				No. of recombinants of each genotype on selective media supplemented with				
Genotypes of selectable progeny				Phenylalanine + uracil	Phenylalanine methionine	Serine + uracil	Serine + methionine	Average frequency
Cross A								
<i>phe</i>	<i>ura</i>	<i>ser</i>	<i>met</i>					
+	+	+	+	1	0	0	1	1
<i>phe</i>	+	+	+	1	1	—	—	1
+	<i>ura</i>	+	+	124	—	133	—	129
+	+	<i>ser</i>	+	—	—	0	0	0
+	+	+	<i>met</i>	—	109	—	117	113
<i>phe</i>	<i>ura</i>	+	+	10	—	—	—	10
+	+	<i>ser</i>	<i>met</i>	—	—	—	0	0
<i>phe</i>	+	+	<i>met</i>	—	33	—	—	33
+	<i>ura</i>	<i>ser</i>	+	—	—	0	—	0
Cross B								
<i>phe</i>	<i>ura</i>	<i>ser</i>	<i>trp</i>	Phenylalanine + uracil	Phenylalanine + tryptophan	Serine + uracil	Serine + tryptophan	Average frequency
+	+	+	+	1	7	2	0	3
<i>phe</i>	+	+	+	22	13	—	—	18
+	<i>ura</i>	+	+	102	—	185	—	144
+	+	<i>ser</i>	+	—	—	0	1	1
+	+	+	<i>trp</i>	—	148	—	192	170
<i>phe</i>	<i>ura</i>	+	+	67	—	—	—	67
+	+	<i>ser</i>	<i>trp</i>	—	—	—	0	0
<i>phe</i>	+	+	<i>trp</i>	—	27	—	—	27
+	<i>ura</i>	<i>ser</i>	+	—	—	0	—	0

(b) Analysis of the frequencies of cotransfer of selected and non-selected markers

	Phenylalanine + uracil	Phenylalanine + methionine or tryptophan	Serine + uracil	Serine + methionine or tryptophan
Cross A				
Counterselected marker	(<i>ser</i>)	(<i>ser</i>)	(<i>phe</i>)	(<i>phe</i>)
Selected marker	<i>met</i> ⁺	<i>ura</i> ⁺	<i>met</i> ⁺	<i>ura</i> ⁺
Non-selected markers	<i>ura</i> ⁺ : 2/136 (2%) <i>phe</i> : 11/136 (8%)	<i>met</i> ⁺ : 1/143 (1%) <i>phe</i> : 34/143 (24%)	<i>ura</i> ⁺ : 0/133 <i>ser</i> : 0/133	<i>met</i> ⁺ : 1/118 <i>ser</i> : 0/118
Cross B				
Counterselected marker	(<i>ser</i>)	(<i>ser</i>)	(<i>phe</i>)	(<i>phe</i>)
Selected marker	<i>trp</i> ⁺	<i>ura</i> ⁺	<i>trp</i> ⁺	<i>ura</i> ⁺
Non-selected markers	<i>ura</i> ⁺ : 23/192 (12%) <i>phe</i> : 89/192 (46%)	<i>trp</i> ⁺ : 20/195 (10%) <i>phe</i> : 40/195 (21%)	<i>ura</i> ⁺ : 2/187 <i>ser</i> : 0/187	<i>trp</i> ⁺ : 1/193 <i>ser</i> : 1/193

(c) Mapping of mutations by co-transfer percentages



For both crosses the mating mixture was plated undiluted on the four selective media; colony counts on the four media were approximately equal. 150 colonies from cross A and 200 from cross B were picked from each selective medium and characterised for non-selected markers.

We tested RP4, which has been used for conjugation studies in *R. leguminosarum*⁷, R68.44 and R68.45 for their ability to promote recombination in crosses between *R. leguminosarum* strains, using a membrane mating procedure¹². In these crosses equal numbers of donor and recipient bacteria were sucked on to Millipore membranes and incubated overnight on complete medium plates. The bacteria were then washed off in distilled water and between 10^9 and 10^{10} bacteria were plated on each selective medium. The transfer frequencies of RP4, R68.44 and R68.45 (per recipient) were about 10^{-2} . The R factors were introduced into the *R. leguminosarum* strains by crossing them with appropriate R^+ *E. coli* donors¹². All *R. leguminosarum* strains were derivatives of the wild-type strain 300¹³ which is able to form normal nitrogen-fixing nodules on the pea (variety Wisconsin Perfection).

RP4 and R68.44 were equally inefficient in promoting chromosome transfer in *R. leguminosarum*. For both, the recombination frequency of approximately 10^{-9} per recipient was similar to the reversion rates of the markers being examined and was quite unsuitable for mapping purposes, though a low frequency of true recombination was proved by the recovery of some progeny carrying auxotrophic markers of both parents¹². R68.45 was about 100 times more efficient and crosses using it routinely produced enough recombinants for linkage analysis. Four-factor crosses of the type described by Hopwood^{14,15} for mapping in *Streptomyces* were used because they facilitate recovery of nine of the 16 possible combinations of parental markers by plating on four different selective media and therefore provide a considerable amount of information.

In such crosses, two of which are shown in Table 1a, four of the recovered genotypes differ from one parent or the other by a single marker and the other five differ from both parents by two markers. Of these five, four (the last four in each tabulation) represent two pairs of complementary genotypes. It is clear that: (1) the genotypes differing from

Segregation of *str* and *rif* as non-selected markers in further crosses indicated that neither was closely linked to *ura-14*, *met-14* or *phe-1* and that they appeared to be linked together. A selective analysis of their segregation showed that they were closely linked: selection for transfer of *rif* to the R^- parent nearly always led to loss of the recipient *str* marker (Table 2). In *P. aeruginosa*¹⁶, *Bacillus subtilis*¹⁷ and *Streptomyces coelicolor*¹⁸, *rif* and *str* genes are also closely linked; in the latter two organisms rifampicin resistance mutations mapping near *str* have been shown to be in the RNA polymerase gene^{17,18}.

The mutation *trp-16* is closely linked to *phe-1* (Table 1) but *trp-12* showed no linkage with *met-14*, *phe-1* or *ura-14*; this indicates that, unlike *E. coli* or *Bacillus subtilis*, but like *S. coelicolor*¹⁹ and *P. aeruginosa*²⁰, *R. leguminosarum* does not have a single tryptophan operon. This observation is confirmed by data from crosses involving a *R. leguminosarum* donor carrying a derivative of RP4 that mediates the transfer of *trp*⁺-14 about 100 times more frequently than RP4 (J.E.B., unpublished results). This donor transfers *trp*⁺-14 and *trp*⁺-12 at the same high frequency, but *trp*⁺-16 at the frequency expected for a wild-type RP4 donor.

In crosses between *P. aeruginosa* strains, R68.45 has multiple sites of origin of chromosome transfer and mobilises fairly short fragments of donor chromosome (about 10–30 min of the *P. aeruginosa* map)⁹. Both these properties of the plasmid seem to apply in *R. leguminosarum* crosses. When selection is for the transfer of markers not showing linkage with *phe-1*, such as *str*-37 or *rif*-74, they are found to be transferred as frequently as *phe*⁺-1. Analysis of recombinants shows that, while fairly long fragments can be transferred, most recombinants arise from the inheritance of smaller donor fragments (Table 1). R68.45 transfers at a lower frequency between *R. leguminosarum* strains than between *P. aeruginosa* strains (about 1% and 50% respectively) and this is correlated with the lower frequency of recombination observed in *R. leguminosarum*. But the problem of distinguishing spontaneous revertants from recombinants when analysing progeny on selective media was facilitated by the low frequency of R factor transfer. Spontaneous revertants have a 1% chance of becoming R^+ and therefore if true recombinants are being formed (for markers with suitably low reversion rates) they should be R^+ . Routinely this was observed; fewer than 5% of colonies on selective media were R^- .

Why is R68.45 able to mobilise *P. aeruginosa* and *R. leguminosarum* chromosomal genes so efficiently when RP4 and R68.44 are so inefficient in *R. leguminosarum*? RP4 (RP1, R1822)²¹ is a poor sex factor in *P. aeruginosa*⁹ but a good one in *Acinetobacter*¹¹. The donor properties of this plasmid are therefore strain (or species) specific and *R. leguminosarum* strain 300 behaves like *P. aeruginosa* strains PAO and PAT. R68.44 would be expected to behave like R68.45 in *R. leguminosarum*. However R68.44 is an unstable donor in *P. aeruginosa*⁹ and presumably lost this property during its transfer from *P. aeruginosa* to *E. coli* and then to *R. leguminosarum*. R68.44 and a number of similar plasmids were isolated by testing *argB*⁺ recombinants in *P. aeruginosa* for enhanced donor activity⁹. The testing of recombinants arising from the transfer of markers from other regions of the chromosome did not yield such donors. Therefore it is likely that R68.44 (and hence R68.45) arose as the result of an interaction between the R factor and *P. aeruginosa* chromosome, rather than the selection of an already existing plasmid mutant. It seems unlikely that R68.45 functions as an improved sex factor in *P. aeruginosa* by virtue of being analogous to an F-prime in *E. coli*. If it were an R-prime it should only have one origin of high frequency transfer in *P. aeruginosa* and would not be expected to function in *R. leguminosarum*. R68.45 also promotes chromosome transfer in *E. coli* K12 strains more

Table 2 Segregation of *rif* and *str*

Cross	No. of R^+ colonies selected on medium containing phenylalanine, cysteine, tryptophan and rifampicin			
	<i>phe</i>	<i>trp</i>	<i>rif</i>	<i>str</i>
Cross B				
<i>ura-14 met-14 rif-74</i> (R68.45)				
×				
<i>phe-1 trp-12 str-37</i>			2	65
Cross C				
<i>ura-14 met-14 rif-74</i> (R68.45)				
×				
<i>phe-1 cys-7 str-77</i>			2	86

Cross mixtures were plated undiluted and all colonies were picked and characterised for non-selected markers. R^- *phe cys str rif* and *phe trp str rif* colonies were assumed to be spontaneous mutants; there were 12 for cross B and none for cross C.

the R^- parent by a single marker were much more frequent than those differing from the R^+ parent by a single marker; (2) one member of each complementary pair of genotypes was much more frequent than the other. Thus we conclude that, as with $F^+ \times F^-$ or $R^+ \times R^-$ crosses in *E. coli*, transfer was polarised from the plasmid-carrying parent and we can analyse the data for linkage on this assumption. On each plating medium, one donor marker is selected, one is counterselected and the other two are non-selected. The frequencies of the donor non-selected alleles are tabulated (Table 1b). There is no significant transfer of *ser-1*, which is therefore unlinked with the other four; therefore we can analyse their linkage on the media on which *ser-1* is counterselected, without complications due to their possible linkage with the counterselected allele. The other four markers show linkage and the percentage cotransfer frequencies indicate a probable map order *met-14-trp-16-phe-1-ura-14* (Table 1c).

efficiently than R68 (J.E.B. unpublished observations). It seems likely therefore that R68.45 was formed by the pick-up of a *P. aeruginosa* DNA sequence(s) that enables it to interact with the chromosomes of genetically distinct hosts. Insertion sequences are involved in "illegitimate" recombination events^{22,23} and may well be implicated here. Since R68.45 can function as a good sex factor in *R. leguminosarum*, *P. aeruginosa*, *P. putida* and *E. coli* it may be useful in developing genetic studies in other Gram-negative bacteria that are hosts for P group R factors.

We hope that development of the recombination system described here will open the way to a genetic analysis of mutations affecting the nitrogen-fixing symbiosis between *R. leguminosarum* and the pea. It is significant that the majority of the auxotrophic and antibiotic-resistant mutations used as markers did not prevent the formation of effective nodules by multiply marked R⁺ or R⁻ strains (J.E.B., unpublished results) and so should not interfere with the study of relevant mutations.

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Note added in proof: Since completing this work, we have learnt that Meade and Signer²⁴ have demonstrated linkage between chromosomal genes in *R. meliloti* strain 2011, using RP4 as a sex factor.

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to small non-electrolytes. Examination of tight junctions with the freeze-fracture technique has revealed the presence of a network of intramembranous fibrils⁴ or strands^{5,6}. Some evidence suggests that these strands constitute the sealing component of the junction⁷, and Claude and Goodenough⁸ correlated the transepithelial permeability of a given epithelium with the number of strands in the tight junction network. Artificial methods of altering transepithelial permeability (hypertonic solutions applied to the mucosal side of the epithelium) have been used to investigate changes in transepithelial electrical resistance⁹, and freeze-fracture morphology⁹ or both¹⁰. Results from these studies do not consistently support the above correlation. An alternative approach is described here, in which the freeze-fracture technique has been used to study tight junctions of the sheep choroid plexus during foetal development. The results obtained suggest that there is no change in the ultra-structural features of tight junctions which have previously been suggested to correlate with transepithelial permeability, in spite of considerable changes in permeability during the developmental period studied¹¹⁻¹³.

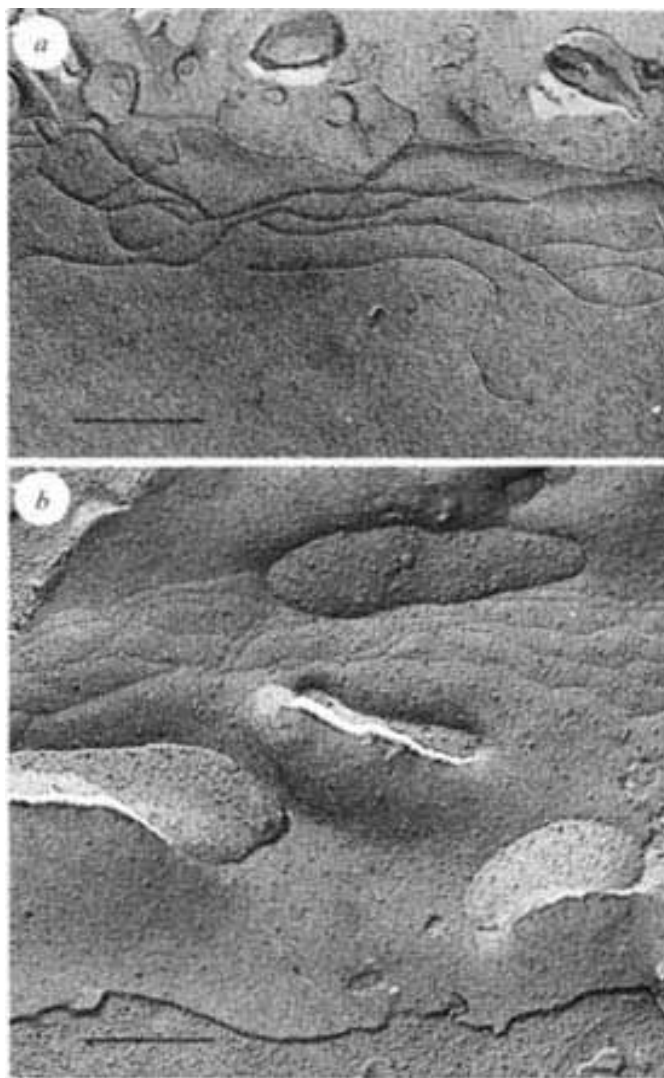


Fig. 1 Freeze-fracture replicas of a choroid plexus epithelial cell from *a*, a 40-d and *b*, a 125-d gestation foetal sheep. *a*, The fracture has exposed a large area of tight junction towards the apex (cerebrospinal fluid surface) of the cell. There are at least four strands running roughly parallel to the apical cell surface. *b*, Note the similar number of strands compared with 40-d (*a*) and that the junctional depth is at least as great at 40-d as at 125-d. Bar indicates 0.5 μ m.

Lack of correlation between tight junction morphology and permeability properties in developing choroid plexus

ELECTRON microscopical investigations of tight junctions (zonulae occludentes) using marker substances, have indicated that in some tissues these junctions may represent an extracellular pathway for transepithelial movement of certain small solutes¹ and also restrict the movement of larger solutes². The electrical resistance across some epithelial tissues has been used as a measure of the degree of permeability of the tight junctions to ions³, and it was suggested that junctions that are "leaky" to ions are also leaky

Freeze-fracture replicas were prepared from sheep foetal choroid plexuses at 40, 45, 60 and 125 d of gestation using methods described previously¹⁴. Figure 1a shows a replica of a fracture through the apical region of the choroid plexus epithelium of an early sheep foetus (40 d gestation); it shows the cell membrane faces of adjoining cells. Below the level of the microvilli are seen the linear ridges and complementary linear grooves which constitute the tight junction. Figure 1b shows a replica at 125 d gestation; its appearance is not obviously different from that at 40 d. Similar results were obtained at 45 and 60 d gestation. To test whether permeability of non-electrolytes and establishment of ionic gradients might correlate with strand number and/or junctional depth, detailed studies of a large number of tight junctions at different foetal ages have been carried out (K.M. and N.R.S., unpublished). Measurements of the minimum number of strands and the mean junctional depth were made as described by Claude and Goodenough⁶. Freeze-fracture replicas of more than 400 areas of lateral-apical cell membrane have been examined. Even in the youngest foetuses the choroid plexus intercellular tight junctions contained several continuous strands in all cases. At 40 d gestation the minimum number of strands was 3.64 ± 1.12 and the mean junctional depth was $0.34 \pm 0.17 \mu\text{m}$ (s.d., $n=121$). At 125 d gestation the number of fibrils (3.45 ± 1.13 ; s.d., $n=49$) was not significantly different, but the junctional depth ($0.28 \pm 0.14 \mu\text{m}$; s.d., $n=49$) was significantly less. It has been shown that at these gestational ages, the thin section electron-microscopic appearance of the tight junctions was similar (C. A. N. Evans, D.H.M., K.M., J. M. Reynolds, M. L. Reynolds and N.R.S., unpublished).

Studies of the penetration of materials from blood into brain and cerebrospinal fluid (CSF) in sheep foetuses of the same gestational ages as those examined in the present study have shown considerable changes in the permeability characteristics of the system during foetal development. For example, lipid-insoluble molecules of small molecular weight (erythritol, sucrose and inulin) as well as albumin, penetrate from blood into CSF at a rate and to an extent which may suggest unrestricted passive diffusion at 60 d; in contrast, there is considerable restriction by 125 d, at which stage the mean pore radius was estimated to be about 1 nm (unpublished). Foetal development of CSF-plasma electrolyte gradients has been discussed previously¹¹ and the gradients for individual ions were shown to appear at very different stages in the order magnesium, chloride, potassium, calcium. The concentration of protein in early foetal CSF is very high, for example 500 mg per 100 ml in 55-d sheep foetuses. At least part of this high level seems to be caused by a mechanism which transports specific proteins (for example, α -foetoprotein and transferrin) from blood into CSF early in gestation but not later when the CSF concentration of protein has fallen to near adult levels (50 mg per 100 ml)¹³.

Since tight junction strand number as revealed by freeze fracture does not change significantly during a major part of gestation, this cannot account for the observed alterations in the permeability properties. From the wide variety of epithelia studied with the freeze-fracture technique it is clear that there are important exceptions to the proposed correlation of permeability with fibril (strand) number (ref. 10 and refs therein). Additional evidence against this correlation comes from recent experiments¹⁶ which showed that in the toad bladder treated with hypertonic lysine there was a marked decrease in transepithelial resistance, but no significant change in either fibril number or mean depth of the tight junctions.

It may be that some other feature of tight junctions not revealed by electron microscopy could account for some of these changes in permeability. Both the molecular structure of the junctional membrane and the biochemical composition

of the material enclosed by the junction fibrils might be important for passive ion transport. Nevertheless, one type of extracellular pathway through tight junctions could not account for all our permeability data for the blood-CSF barrier. For example, as mentioned above, there is penetration of albumin from blood into CSF in the 60-d foetus. Such a large pathway, were it present within choroid plexus tight junctions, would be well within the resolution of our electron-microscopical observations. It seems more likely that protein and non-electrolyte penetration and perhaps some type of ion transport involves a quite different route, that is, a transcellular one, consisting of a tubulovesicular system. An intracellular system of tubules and cisternae of endoplasmic reticulum has recently been implicated in transcellular transport of protein¹⁴ and non-electrolytes (C. A. N. Evans, D. H. M., K.M., J. M. Reynolds, M. L. Reynolds and N.R.S., unpublished) in foetal choroid plexus as well as in active sodium transport in frog skin¹⁵.

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Errata

The order of the authors of the article "Release and metabolism of substance P in rat hypothalamus" (*Nature*, **264**, 81; 1976) should have been T. Jessell, L. L. Iversen and I. Kanazawa and not as printed.

In the article "Stratospheric aerosols and climatic change" (*Nature*, **263**, 551; 1976) the order of the authors' names was changed and should have read J. B. Pollack, O. B. Toon, A. Summers, B. Baldwin, C. Sagan and W. Van Camp.

In the article "Polymers for the sustained release of proteins and other macromolecules" by R. Langer and J. Folkman (*Nature*, **263**, 797; 1976) the units on the abscissa in Fig. 1 should be hours.

matters arising

A precise definition of chaos

MAY¹ has recently reviewed work on the dynamics of simple recurrence relations. If $x_{n+1} = f(a; x_n)$ where a is a parameter, the system exhibits different behaviours for different values of a . In many cases, there is a range of behaviour characterised by three properties:

- (1) Not all solutions are periodic;
- (2) Asymptotic stability need not obtain;
- (3) Cycles of all periods may occur.

Li and Yorke² show that (1), (2) and (3) necessarily follow if a 3-cycle exists and these authors term such behaviour 'chaotic'. They also show that a 5-cycle may exist without there being a 3-cycle.

The Ukrainian author Sharkovsky³ proves a more general result, which deserves to be more widely known. Let $n_1 < n_2$ if $n_1 \neq n_2$ and the existence of an n_1 -cycle implies the existence of an n_2 -cycle. Sharkovsky shows that

$$\begin{aligned} 3 < 5 < 7 < 9 < \dots \\ < 2.3 < 2.5 < 2.7 \dots \\ < 2^2.3 < 2^2.5 < 2^2.7 < \dots \\ < 2^3.3 < 2^3.5 < 2^3.7 \\ < \dots < \dots 2^3 < 2^2 < 2 < 1 \end{aligned} \quad (1)$$

He also demonstrates that there exist recurrence relationships with $(2m+1)$ -cycles but not with $3, 5, \dots, (2m-1)$ -cycles, for any natural number $m = 2, 3, 4, \dots$

In a subsequent paper⁴, Sharkovsky classifies the behaviour of the system in terms of the topological properties of the set C of all points belonging to cycles. If C is a closed set, then all initial values x_0 lead to asymptotically periodic behaviour. This occurs when there are only finitely many cycles (necessarily 2^n -cycles by relation (1)), and in some instances where there are infinitely many cycles all with orders of the form 2^n . If C is not a closed set the number of x_0 not leading to asymptotic periodicity is uncountably large. This occurs whenever there are cycles other than 2^n -cycles and, in some cases, where all the 2^n -cycles occur but only those.

In the first of these instances, requirements (1) and (2) are satisfied, but not necessarily (3). It is not known whether this is true in the second instance, which, however, we conjecture to be structurally unstable to changes in the value of a .

We suggest that requirements (1) and (2) be regarded as defining chaos, but that (3) not be regarded as necessary. This would entail some divergence from the nomenclature of certain authors (for

example, May¹), but it is a consistent convention and one in accord with everyday usage.

A detailed, though still incomplete, list of Sharkovsky's papers is provided by Sibirsky⁵. We also draw attention to the work of Barna⁶, Berg⁷ and Coppel⁸.

We thank Mr W. A. Coppel for his help in supplying some of the reference material. A more complete account of this work will appear elsewhere.

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Heat-transfer enhancement with electric fields

CHANGES in heat transfer brought about by electric fields, as reported by Asakawa¹, have been observed for many years. The discovery of the phenomenon is generally attributed to Senftleben and Braun² who observed the systematic enhancement of heat transfer in various gases contained between a cylindrical surface and a co-axial wire when electric fields greater than about 42 kV cm⁻¹ were produced at the surface of the wire. The effect was mentioned as early as 1709, however³.

The results reported by Asakawa seem primarily to be the retardation of heat transfer, but in fact seem similar to those expected on the basis of simple inductive heating. It is difficult to quantify heat transfer results with transient experiments, and is generally more informative to rely on steady-state (for example, calorimetric) measurements. Although it is apparently tempting to suggest that the phenomena observed by Asakawa involve material

changes in the material (fluid), there is no evidence for such a conjecture.

We have studied the enhancement of heat transfer in gases by inhomogeneous electric fields in some detail⁴. Our results show that this effect, which, for convenience we call electrocooling, is simply an ionic drag phenomenon (that is, the electric wind of Chattock⁵) in which the convective heat transfer coefficient is proportional to the fourth root of the induced corona current. We derived a theoretical expression which predicts such corona-induced heat transfer within 20% of experimental values for electro-negative gases such as air. As a particular type of forced convection, electrocooling may thus be directly compared to conventional devices such as air jets or blowers.

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Scrotal asymmetry and Rodin's dyslexia

MCMANUS¹ has pointed out that most artists depict the left testicle of man lower than the right, in accordance with the facts². An exception occurs in Rodin's famous sculpture *L'Age d'Airan* where the right testicle of the figure seems (from photographs we have seen) to hang lower than the left. We should like to suggest that Rodin was genuinely confused about left and right. Evidence that Rodin suffered from a specific reading disability, which is commonly associated with left-right confusions³, has been reviewed by Thompson⁴. So badly did the artist do in school during his early years that his father was informed: "He is ineducable. The sooner you put him out to work, the better. But I doubt if he can ever make a living."⁵

Some of the facts about scrotal

asymmetry seem themselves rather confused. According to Chang *et al.*², left-handed people may show a reversal of the usual scrotal asymmetry. Cholst⁶, however, maintains that reversal occurs only in the extremely rare condition of *situs inversus totalis*. Chang *et al.* also report that the right testicle is usually heavier than the left, which seems superficially at odds with the observation that the left testicle is lower¹. The generality of this finding should perhaps be checked, in view of the puzzling discrepancy over the relationship to handedness. Nevertheless there is some support for the weight difference described by Chang *et al.* in a recent report that the right human gonad normally develops more rapidly than the left⁷.

We have recently suggested that there is a general tendency for growth gradients to favour the left-hand side^{8,9}. Examples are seen in the temporal planum of the human brain; the habenular nucleus in the brains of amphibia; the control of birdsong by the left hypoglossal nerve; the cervical vertebrae of chickens; the greatly elongated left tooth of the Narwhal; and the left-sided development of the hydrocoel in echinoderms. The concordance among these asymmetries is intriguing, but we are obliged to admit that the faster development of the right gonad in mammals tends to restore parity to the situation.

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the specificity of the type of lymphocytes involved. They showed that PLA from cobra venom induced the ability to form E rosettes in B-type human peripheral lymphocytes and lymphoid cell lines. In contrast, PLA from bee venom and bovine pancreas were ineffective.

I have used PLA from *Crotalus adamanteus* venom, partially purified on Sephadex G-200 and assayed by a lecithin breakdown test^{2,3}. Incubation of this preparation with human lymphocytes at 37 °C for 1 h resulted in a marked decrease in E-rosette formation. Titration experiments showed that the maximum inhibitory effect was produced with PLA at 1 mg ml⁻¹. The effect of this enzyme preparation was resistant to heating to 100 °C for 10 min, but it was not active at 4 °C. It was non-toxic to human lymphocytes and erythrocytes as measured by release of radioactive chromium. Lymphocytes could recover the ability to form E rosettes ~ 8 h after removal of the enzyme on further incubation at 37 °C. Furthermore, PLA treatment of human spleen membranes released material which could block erythrocytes^{2,3}.

These earlier results conflict with those of Hanaumi *et al.*¹, but the discrepancy may be a result of the different sources of enzyme¹. In addition, Zwaal *et al.*⁴ have shown that PLA purified from cobra venom and bee venom does convert lecithin to lysolecithin, in contrast to that purified from bovine pancreas. They also showed that *in situ* lysolecithin does not cause lysis (of human red cells).

This difference between results is striking. I showed that PLA-treated lymphocytes do not form rosettes, probably because of removal of the E receptors which can be re-expressed by the lymphocytes after removal of the enzyme. This may provide a method of solubilisation of the receptors for chemical characterisation. Hanaumi *et al.* showed however, that the E receptor is resistant to their PLA preparation and suggested that there are E receptors on human B cells which are exposed by PLA, although they were unable to show that neuraminidase treatment unmasked these receptors on B-type lymphoid cell lines, in contrast to peripheral B lymphocytes⁵.

This difference has practical importance for the further study of E receptors.

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HANAUMI AND KUMAGAI REPLY—We thank Chapel for her comments¹ on our report concerned with the nature of the receptor for sheep erythrocytes on human lymphocytes and lymphoid cell lines². We too have tested the effect of PLA partially purified from the venom of *Crotalus terrificus*, which is one of the Crotalidae snakes, but not *C. adamanteus* used by Chapel³ on the lymphocytes and lymphoid cell lines. The enzyme preparation (specific activity; 14.7 μmol min⁻¹ mg⁻¹ as assayed using phosphatidylethanolamine from *Escherichia coli*) at 40 μg ml⁻¹ did not induce the ability to form rosettes on lymphoid cell lines. In contrast, incubation of this preparation with human lymphocytes at 37 °C for 30 min showed a marked decrease in E-rosette formation, similar to that observed by Chapel with *C. adamanteus* PLA. Our experiments showed, however, that this treatment simultaneously degrades almost all the receptors for immunoglobulin Fc and complement C3 present on the non-treated lymphocytes and lymphoid cell lines, and also that it strips off the surface immunoglobulins⁴.

In contrast to the Crotalidae enzyme preparation, PLA highly purified from *Naja naja* venom induced the ability to form rosettes, but had no effect on the immunological receptors mentioned above. When the cells treated with PLA, even at concentrations greater than 640 μg ml⁻¹, were incubated at 37 °C in medium containing a foetal calf serum, they grew exponentially at the same rate as did non-treated cells, and the ability to form rosettes disappeared from the cell with time⁴. During these experimental periods no markers other than E receptors changed.

Thus, we agree with Chapel's arguments that among the effects of PLAs from different sources may exist a striking difference which has practical importance for the further study of E receptors. We should emphasise, however, that the PLAs of *Naja naja* and bee venom, though the latter exhibited only a weak activity on the human lymphoid cells, were specifically active in inducing the ability to form rosettes of the cells.

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Rosette formation by human lymphocytes

HANAUMI *et al.*¹ point out the need to elucidate the nature of the receptor for sheep erythrocytes (E) on human T lymphocytes. They have examined the effect of phospholipase A (PLA) on E-rosette formation in a study of

reviews

Chemical kaleidoscope

Peter Farago

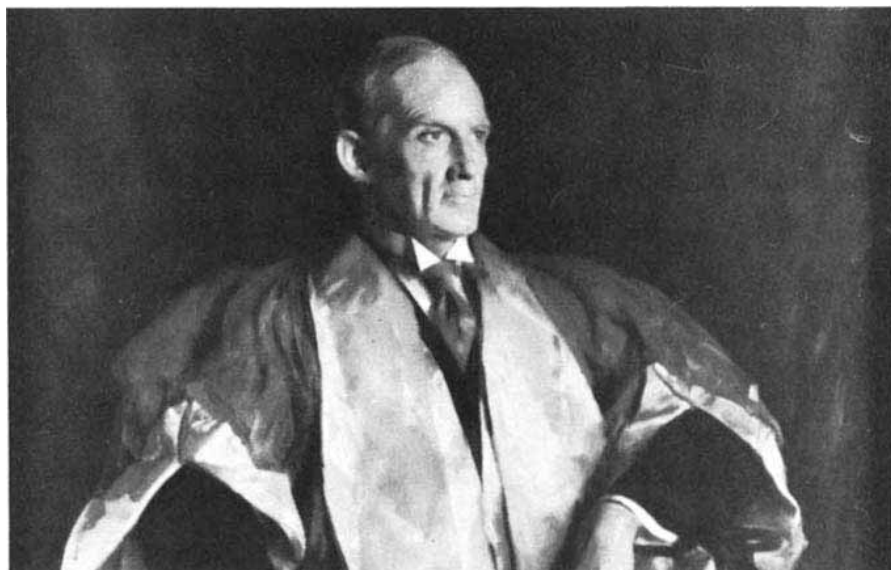
Memoirs of a Minor Prophet: 70 Years of Organic Chemistry. By Sir Robert Robinson. Pp. viii+252. (Elsevier: Amsterdam, Oxford and New York, 1976.) Dfl.49.50; \$18.95.

I LAST met Sir Robert Robinson a few months before his death in 1975. He looked very old. His sight had become weak and he moved with difficulty. But, sitting behind the big polished desk in his office high above the Thames, he suddenly became animated as he described the research he was still directing. His judgements on people and events showed none of the hazy mellowness to which an 88-year-old is surely entitled.

Robinson has been described by Lord Todd as "one of the few men of science to illumine by their own genius a wide variety of areas in their chosen subject" (*Chem. Brit.*, 11, 296, 1975). Within the international community of chemists, there were, and are, many deeply attracted by Robinson both as a man and as a scientist; yet one must record different reactions also. Nobody working in organic chemistry during the period 1915 to the mid-1950s could remain uninfluenced.

His book is a series of episodic recollections of people, places and scientific work, from early childhood to appointment to the Waynflete Chair of Chemistry at Oxford in 1930. One can compare it to the autobiographical writings of another great chemist, *Aus meinem Leben: von Arbeit, Musse und Freuden*, by Richard Willstätter (edit. by Arthur Stoll, Verlag Chemie, Weinheim, 1949), a tragic and human document about the fate of an outstanding scientist during the cataclysm of 20th century Europe. *Memoirs of a Minor Prophet* does not pretend to those heights because, one suspects, what really interested its author were ideas and things, rather than the human condition. As has been said about one of his great protagonists, he talked to molecules. If he suffered anguish, if he lay awake at night wrestling with the agonies of personal decisions, his book does not tell us about it. Emotions seem to have been fully engaged only with the ideas, substances and practitioners of science.

The autobiography presents a kaleidoscope of chemical ideas and personal views in the context of the first 30



years of this century, when organic chemistry was going through one of its most creative periods. Starting from a comfortable West Country middle-class background, the future Sir Robert passed through a Moravian Church school to Manchester University where H. B. Dixon, a pupil of Vernon Harcourt, was senior professor. Robinson entered the laboratories of W. H. Perkin, Jr, as a research student and started his academic career in the newly established Chair of Pure and Applied Organic Chemistry at Sydney in 1912. In the next 16 years he held five other appointments. Returning to a Chair in Liverpool in 1915, he spent a short, uncomfortable interlude with British Dyes Ltd in Huddersfield, followed by a Chair in St Andrews in 1921.

He returned to Manchester as Professor of Organic Chemistry in 1922 and transferred to University College, London, in 1928. The present book ends on the eve of his departure for Oxford. At each station of his progress there are descriptions of some colleagues and of research work in progress. The point of view is explicitly personal, and not all his contemporaries or those who followed would be expected to agree with it.

This was a most important period not only for science but also for scientists, whose contributions to the social fabric were being generally recognised; but there seem to have been few bridges between the worlds of university, in-

dustry and government the author describes. Of Robinson's contributions both as a theoretician, a practical chemist, and later as an industrial consultant, there can be no doubt. Whatever controversies surrounded some of the theoretical work, whole new fields of organic chemistry were explored by Robinson and his collaborators, laying the groundwork for spectacular insights not only in chemistry but also in many of the life sciences.

There are indications of all these strands in *Memoirs*, but the themes find their context only in terms of the author's personal impressions. Organic chemists will find vignettes of people, occasions and ideas which show the origin of a number of current developments in the science. They might usefully compare their present pre-occupations with those of Robinson's generation, observing carefully not only those ideas treated in the book but those which are absent. For those who are not chemists, the need for highly specialised knowledge of the subject will create a formidable deterrent to understanding. But even for them *Memoirs* does dispose of, yet again, the mythical unemotional scientist, the desiccated calculating engine.

Beautifully produced and not unreasonably priced, the book is utterly disgraced by the lack of subject and topic indices. □

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Diversity of the ecosystem

Ecological Diversity. By E. C. Pielou. (Wiley-International: New York and London, February 1976.) \$16; £8.

It is perhaps idle to speculate on what future generations of ecologists will, with their hindsight, deem to be the important developments of the mid-1970s. One area of ecology, however, that almost certainly will not go unheeded is the complexity-stability problem: not that this is a problem which originated in the mid 1970s but rather that there has been a plethora of approaches to the problem during the past four or five years. The arguments will remain, but one aspect of the problem that has occupied several ecologists is the measurement of the diversity of the ecosystem. Diversity, or complexity, can be measured virtually instantaneously whereas stability, the other side of the problem, can only be measured by observation over a long period of time.

Professor Pielou's book is thus a timely account of the one side of this ecological problem. The introductory chapter includes a brief review of the indices for measuring diversity and

equitability, and stresses particularly the different conceptual treatment of fully censured, as opposed to sampled, communities. The next three chapters provide a useful survey of the models and distributions that have been proposed to account for the number of individuals within the species of a community, and of testing hypotheses about species abundance. Again a distinction is made, being between the type of model based on ecological considerations (for example, the broken stick and niche pre-emption models) and the statistical model (for example, the negative binomial distribution).

The book is concluded with a series of four chapters which could be described as applications and 'odds and ends'. One of these chapters deals with spatial mosaics, with several pages devoted to the use of Markov-type matrices for analysing sequences along a transect, but in an example the categories used are 'bare ground', 'herbs', 'grasses and sedges' and 'tree seedlings', which seem to be a far cry from the precision of the first four chapters. Two other chapters contain a consideration of eight "determinants of diversity"—such factors as the diversity on small islands or the co-existence of potentially competing species. It seems here as if Professor

Pielou is trying to discuss a few areas in which she has personally worked, and thus the coverage of the subject is far from complete (for example, ecological succession is probably one of the more common factors affecting diversity, but this only receives a passing reference in a section dealing with the geological timescale).

Ecologists should take note of this book, particularly as a more rigorous assessment of diversity is likely to be required in approaching the complexity-stability problem. I very much doubt if they will. The extremely mathematical style will put many readers off, which is more the pity since some of the ideas of the last four chapters could usefully be more widely applied and developed. The somewhat obscure style of writing—for example, the third property of diversity indices in section 1.2 which is poorly described—is very frustrating for the reader. But the subject matter shows that there is still a lot of research to do in this field, and I hope the book will point out current deficits in our knowledge.

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Xenopus as a model system

From Egg to Adolescent: Xenopus—A Model for Development. By Louie Hamilton. Pp. xi+78. (English Universities Press: London, March 1976.) £3.45.

AMPHIBIANS have long been one of the most popular organisms in embryological laboratories, and in recent years *Xenopus laevis* has become the most widely used amphibian species. Its first important application was in nuclear transplantation, for which it was remarkably, perhaps fortuitously, well adapted. These experiments provided us with the strongest available evidence that all the cells of an organism contain identical genes, and in turn that development involves the continuous regulation of genetic activity. Coming on to the scene as late as it did, it is natural that *Xenopus* has made its main contributions to molecular biology, and to the infant science of molecular embryology. Moreover, certain biologists moved from developmental studies to the chemical dissection of gene structure, without changing their chosen organism. The result is that more is now known about the structure of some genes of *Xenopus* than of any other higher organism.

All of this must colour our expectations in reading Dr Hamilton's book. The reader will find a valuable summary of the main morphological events in the development of *Xenopus*, with very brief references to attempts at analysing these processes. There are many good pictures, especially electron micrographs, but the text suffers in general from a lack of diagrams that would help the reader to follow the complex morphological processes by which the vertebrate body is formed. It is, for example, very difficult to follow the formation of the nervous system in the absence of diagrams.

Nevertheless, the elementary student will find the description of development useful, and from time to time he will find his interest titillated by references to experimental embryology. Sadly, the book will not satisfy any interest it arouses because so few references are given. Surely the corpus of work on *Xenopus* justifies more than thirty-seven references. The molecular aspects of development are combined with nuclear transplantation in a chapter only four pages long, and with only two references. One feels that *Xenopus* deserves a little more! **H. R. Woodland**

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Proteases and biological control

Proteases and Biological Control. (Cold Spring Harbor Conference on Cell Proliferation, Vol. 2.) Edited by E. Reich, D. B. Rifkin and E. Shaw. Pp. x+1021. (Cold Spring Harbor Laboratory: Cold Spring Harbor, New York, 1975.)

THE very many different ways in which proteases contribute to biological control systems is the strongest single impression given by this conference report. Thus, although proteolytic enzymes have been studied for more than three-quarters of a century, it is only recently that it has become apparent how many different control mechanisms depend on these enzymes.

The work of Bayliss and Starling (1902) on the conversion of trypsinogen to trypsin by enterokinase is mentioned as an early example (K. A. Walsh). The first section on structure-function relationships also contains a valuable contribution from H. Neurath on limited proteolysis and zymogen activation. In the report of a meeting devoted to proteases and biological control, it is hardly surprising to find sections on coagulation, complement and kinins, and fibrinolysis. All, however, are excellent and have given the individual contributors the opportunity both to present recent results and to review their special fields.

The more fundamental aspects of protease action are made more comprehensible by a contribution on the specificity of proteinases toward protein substrates (J. S. Fruton). Also contributing towards an understanding of physiological control systems is the section on proteinase inhibitors in which the roles of both α_1 antitrypsin and α_2 macroglobulin are discussed. A number of rather disparate contributions appear in a section entitled Cellular Aspects of Proteinase Action. In one mainly devoted to protein turnover in animal cells, R. T. Schimke raises some fundamental question—such as the reason why proteins undergo continuous degradation *in vivo*. As he points out, a partial answer may be the possibility that about 15% of all proteins contain substitution errors incurred during synthesis. Another important reason for general turnover is also considered, that in its absence, the degradation of particular proteins, needed as part of many physiological changes, would require a set of specific proteinases as numerous as the proteins to be degraded.

A final section on tumour-associated proteinases contains several interesting contributions—for example on plasminogen activators, which are reported

to increase in concentration by a factor of 50 after neoplastic transformation of primary fibroblasts.

In view of the wide coverage, excellent quality of the individual contributions and rapid developments occurring in the field, the book deserves a warm welcome. **A. H. Gordon**

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Ornament of our times

Handbook of Psychopharmacology. Section 1: Neuropharmacology. Edited by Leslie L. Iversen, Susan D. Iversen and Solomon B. Snyder. Vol. 1: Biochemical Principles and Techniques in Neuropharmacology Pp. xiii+298. \$27. Vol. 2: Principles of Receptor Research. Pp. xii+288. \$27. Vol. 3: Biochemistry of Biogenic Amines. Pp. xii+486. \$37. Vol. 4: Amino Acid Neurotransmitters. Pp. xi+317. \$30. Vol. 5: Synaptic Modulators. Pp. xii+381. \$35.40. Vol. 6: Biogenic Amine Receptors. Pp. xii+307. \$30. (Plenum: New York and London, 1975.)

AFTER listening at a Journal Club to a presentation of the latest contribution of Scandinavian psychiatric epidemiology, Sir Aubrey Lewis was once heard to remark that this was not the sort of work which one could criticise, but was an *opus magnum* simply to be admired. The audience was somewhat astonished, never previously having detected any faltering in their professor's critical appetite. After delivering this one line, however, Sir Aubrey proceeded unabashed in his usual sharp and telling style, and everyone was much re-assured.

This set of six books on basic neuropharmacology represents only the first of three runs which will together comprise the complete *Handbook of Psychopharmacology*. We are therefore at this point looking at only part of a very large design. But even at this early stage we must unequivocally welcome the production of an ornament of our times. Here is a work which bears witness to the excitement and distinction of a branch of scientific investigation which has burgeoned over recent years. There is nothing in these books which is plodding or pedestrian, and nothing of the anxious pedantry which is sometimes the stuff of the *Handbook* which is desperately determined to miss nothing. A galaxy of scientists from many countries have contributed. To attempt the usual re-

view summary of contents would inevitably mean such condensation as to be misleading. It is sufficient to say that every important basic scientific aspect of this subject is authoritatively dealt with, and the subject explored out to its frontiers.

So much in essence for Sir Aubrey's opening statement. Following his example, it would however, be churlish not to offer some critical comment on an enterprise so eminently worthy of serious scrutiny. The largest problem which the editors had to face was obviously that of how to order and sort all the various issues, so as to produce a work which served the likely purposes of many different readers. There can be no absolutely satisfactory way of meeting such a sorting problem—to confine the coherence of one set of themes to entire chapters, other themes may inevitably be scattered between volumes. As a sort of test run, it was therefore interesting to look up the entries for phenothiazines in the index of each of these six volumes—no doubt the drug will again be treated in a later psychopharmacology section. What transpires is that in Volume 1 there is discussion of immunochemical assay (which certainly stands by itself). In Volume 2 the drug gets a mention in the EEG Chapter (blocking of dopaminergic transmission), and in the discussion of structure-activity relationships. Influence on 5-hydroxytryptamine and bearing on neuropharmacological theories of schizophrenia comes in Volume 3, whereas in Volume 4 the effect of chlorpromazine on brain GABA levels, and glycine and glutamate uptake, is discussed; and again the blockage of dopamine receptors. In Volume 5, influence of phenothiazines on brain energetics is dealt with (touching again on 5-hydroxytryptamine and dopamine), and later the inhibition of response to norepinephrine. In Volume 6 aspects of the dopamine questions come up once more, and in discussing the structural modification of phenothiazines and related dopamine receptor-blocking, line illustrations have to be produced which hark back to Volume 2.

There might in the next edition be further room for editorial tidying up, but the design of the enterprise in terms of basic science first and clinical application only later, is probably right and inevitable. It is Sir Aubrey's opening statement which stands.

Griffith Edwards

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Cinnabar elixirs to insulin

Science and Civilisation in China. Vol. 5: Chemistry and Chemical Technology. Part 3: Spagyric Discovery and Invention—Historical Survey from Cinnabar Elixirs to Synthetic Insulin. By Joseph Needham. Pp. xxxv+481: plates 462–476. (Cambridge University: Cambridge and London, May 1976.) £16.

PART 3 of Volume 5 of *Science and Civilization in China* completes the survey of alchemy begun in Part 2 (for review, see *Nature*, 254, 539, 1975). It is more difficult to read than the earlier part because it analyses in detail material which is quite unlike anything with which the Western historian of science is likely to be familiar. Before Needham and his collaborators have been able to get down to any kind of criticism they have needed to examine the material written in a language which differs from Western languages in its basic make-up. It would be an impertinence on the part of any reviewer who, like myself, does not know Chinese, to make any remark on this subject were it not for one important fact about the book itself. Needham goes to a great deal of trouble skilfully to expound these linguistic difficulties and so makes it clear how he set to work and how he solved his problems. In the course of explaining some of the most recent chemistry he has some striking things to say about the different ways Western terms have been adapted to Chinese. His exposition of the language problem becomes a contribution to the history of chemistry.

More than in Part 2 he is concerned with individuals and the extensive record of their work. To follow all of these one needs to know a great deal of chemistry. It will not be easy going for those who do not. As in previous volumes one finds light being shed on Western science. For example, in dealing with Ko Hung, a great alchemist, and his contemporaries, Needham says "How did they keep their heads in the midst of so much religious-magical enthusiasm? How did he manage to make so many true observations of chemical behaviour, and carry out so many interpretable experiments even though he himself could never interpret them? . . . Ko Hung had one eye on the magic, the sacrifices, the Taoist temple liturgies but he kept the other firmly fixed on the real changes and transformations which he observed at his bench and his furnaces". We could apply this analysis to much that is puzzling in Western practice not only in the obviously related field of Western



A woman alchemist, possibly Thai Hsüan Nü, compounding elixirs. Taken from the *Lieh Hsien Chhüan Chuan* (Complete Collection of the Biographies of the Immortals), Ming, about 1580 AD.

alchemy and chemistry, but much other science and proto-science.

The fate of alchemy in China is curiously parallel with its fate in the West. It declined through the Middle Ages and was made the subject of satire. There was even a kind of iatrochemistry as its successor. But instead of a continuous suppression of alchemy by a progressive modern chemistry, there came a gap. Western chemistry was introduced into China bit by bit, to be adapted and assimilated like many other foreign influences, so that in

China, it seems, modern chemistry is the heir of the West, not of the remarkable, indigenous chemistry with which the major part of this book is concerned.

The title suggests that Needham has told a continuous story, but in fact he says plainly that he is not dealing with modern chemistry; the detailed treatment stops at the end of the eighteenth century, with only a few pages on the modern sinisation of terminology, but any more than we are offered would be indigestible.

There is one thing to regret. Needham refers to early illustrations of chemical apparatus but they are not reproduced. Perhaps it was not possible. There is, however, always work to do and we shall look forward to seeing them another time. The general impression is of the predominance of elixir-seeking in Chinese alchemy, and its relationship to the Chinese attitude to length of life as a virtue as well as an advantage. Resemblance to Western alchemy often arises from the fact that the Chinese were dealing with the same chemical substances, so that what they could discover and what they could infer was bound to be the same in the West. But the differences lay in the civilisation itself. Thus, the common ground of science enables one the better to understand what was distinctive in the civilisation. This justifies, if nothing else does, the title of the whole enterprise.

Frank Greenaway

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General relativity for undergraduates

Gravitation and Spacetime. By Hans C. Ohanian. Pp. xiv+461. (Norton: New York, May 1976.) \$18.95.

OVER the past decade there has been an explosion of discoveries, both theoretical and observational, in the subjects of gravity and relativity. Yet curiously there are still very few well written, broadly based and concise introductory books on general relativity which cover these exciting new developments. Perhaps this reflects the tradition of regarding general relativity as primarily a postgraduate and research activity; but increasingly in recent years, universities and colleges are running general relativity courses for undergraduates. This book by Ohanian is ideally suited to such courses as a teaching textbook.

The flavour of the book which emerges, suggests that it is aimed primarily at the physics student. The mathemati-

cal development of the subject matter is competent, and quite adequate for physicists, but perhaps a little sparing for mathematics students primarily interested in the modern techniques of differential geometry and topology, the singularity theorems, and so on.

The book contains much interesting discussion of experimental techniques and results. The later chapters cover modern developments in black hole theory and cosmology. There is little in the way of special relativity.

Many people will find this book useful for reference. Information retrieval is easy, the presentation of the material clear, concise and accurate; students should have little difficulty in finding their way around the subject matter, and exercises are plentiful.

In short, a solid, direct and informative reference and teaching book to be recommended to all students of relativity.

Paul Davies

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Parvoviruses

The Parvoviruses. (Virology Monographs/Die Virusforschung in Einzeldarstellungen, Vol. 15.) By G. Siegl. Pp. iv+109. (Springer: Wien and New York, 1976.) DM56; \$3.86.

THIS excellent little monograph reviews information on known members of the genus *Parvovirus* in the Family Parvoviridae. The volume of data accumulated during the past decade on members of this genus is so great (over 300 major references), and is so complex, that Dr Siegl has wisely concentrated on this genus, leaving information on the genera *Adeno-associated virus* and *Densovirus* to subsequent reviewers.

The intense interest engendered in this newly discovered family of viruses derives from their unique structure, a single-stranded DNA molecule capable of coding for only 3-5 proteins being enclosed in a 20-nm protein capsid. The dynamics of replication of such a single-stranded DNA in eukaryotic cells has provided an exciting challenge, and offered an ideal experimental system to study biochemical processes which occur during replication of DNA viruses.

For each of the nine members of the genus, Dr Siegl traces the work which has led to the finding that these viruses require cellular helper effects to replicate, and in consequence multiply only in actively dividing tissues. Although only two of the nine members have as yet been shown of importance in natural disease, the results of fundamental research on the 'important' members have shown these viruses to have a unique pathogenesis, selectively attacking foetal, neonatal, intestinal and haemopoietic tissue. This finding has in turn stimulated applied virologists to search for disease syndromes to associate with other members of this genus.

Dr Siegl has presented his review in sections, dealing with each virus on a species basis. Although this approach has inevitably led to some duplication of general information, it provides for easy reference by the species specialist, and certainly minimises the confusion associated with some members of the genus. Such complex data are particularly evident for the section on hamster osteolytic parvoviruses, and for those viruses which have been found as contaminants of cell lines. The review given for the porcine parvovirus has been subsequently developed (see Joo and Johnson, *Porcine Parvovirus—A Review*, *Vet. Bull.*, **46**, 653; 1976), and this virus has now been shown to

be a significant cause of natural reproductive failure.

Although information on the significance in nature of the porcine parvovirus and the feline virus have now clearly been established and control measures undertaken, it is disappointing to the reviewer as an applied worker, to see the paucity of information available on the disease significance of the bovine and canine parvoviruses. It is hoped that the future may see as detailed and conscientious a study of the significance of these viruses, and of possible human parvoviruses, as has been presented by the fundamentalists for the rodent viruses.

Although not in any way wishing to detract from the value of the monograph, the reviewer would have welcomed wider discussion and speculation by such a respected authority as Dr Siegl, and a gentle rebuke is included for the publishers in allowing printing of this excellent work without any obvious attempt to correct spelling and grammar.

R. H. Johnson

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Prostaglandins

Prostaglandins: Chemical and Biochemical Aspects. Pp. xiii+252. £9.95. *Prostaglandins: Physiological, Pharmacological and Pathological Aspects.* Pp. x+367. £11.50. *Prostaglandins and Reproduction.* Pp. x+332. £11.50. (Advances in Prostaglandin Research.) Edited by S. M. M. Karim. (MTP: Lancaster, 1975-76.)

As Professor Karim points out in his introduction, it is now impossible to comprehensively cover the entire prostaglandin field in one volume; this latest contribution is accordingly divided into three parts. The complete set comprises more than 20 chapters written with varying degrees of clarity by almost 30 specialists.

Part I surveys current understanding of the role of prostaglandins in reproduction. There is a general discussion of the role of prostaglandins in the reproductive physiology of man (Karim and Hillier), subhuman primates (Kirtan), laboratory (Labhsetwar) and some domestic animals (Flint and Hillier). There are also chapters on the use of prostaglandins to induce labour (Thiery and Amy) and to interrupt pregnancy (Karim and Amy). This volume concludes with an interesting chapter on the use of prostaglandins

and their analogues in animal husbandry (Cooper and Walpole).

Part II deals with the more basic chemical and biochemical aspects of prostaglandin research, beginning with a review by Schneider of chemical synthetic methods for prostaglandins. The methodological information in the chapter on prostaglandin analysis by Salmon and Karim is particularly welcome, as is a thoughtful contribution by Lands and Rome on inhibition of prostaglandin biosynthesis. Sanner and Eakins review prostaglandin antagonists, and in the final chapter Kuehl, Cirillo and Oien attempt to clarify the confused area of prostaglandin-cyclic nucleotide interactions.

Physiological, pharmacological and pathological aspects of prostaglandins are covered in Part III, with interesting chapters on the role of prostaglandins in the central nervous system (Cocconi and Pace-Asciak), autonomic neurotransmission (Hedqvist), ocular (Eakins), gastrointestinal (Bennett), renal and cardiovascular (McGiff and Malik) as well as respiratory (Smith) physiology and blood coagulation (Howie). There is a rather short discussion on prostaglandins in inflammation (Greaves) and a welcome review of prostaglandins in tumours (Karim and Rao); this volume concludes with a useful review of the pharmacology of prostaglandin analogues (Karim and Ganesan Adaikan).

In rapidly developing areas, it is always difficult for reviewers to incorporate the most recent developments. Since 1974, there has been accumulating evidence that in some tissues, a large proportion of prostaglandin endoperoxides are metabolised to non-prostaglandin end-products and that in some cells (for example, platelets) the major biological actions are almost certainly due to the endoperoxides rather than the 'primary prostaglandins' E and F. This important idea was neglected by several contributors. It is regrettable—but unavoidable—that details of the most recent endoperoxide rearrangement products, the thromboxanes, could not be included.

I found this series worthwhile. It contains an abundance of up-dated material and covers much ground never before reviewed. The volumes have been well produced and the standard of the printing and illustrations was generally good, although there were some badly drawn structural formulae in one chapter. These volumes will be a useful addition to any departmental library; the cost of the series puts it beyond the pocket of most individuals.

R. J. Flower

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Agronomy and nutrition of maize

Mineral Nutrition of Maize. By I. Arnon. Pp. 452. (International Potash Institute: Bern-Worblaufen, Switzerland, 1976.)

THIS excellent volume is really two books in one, covering both agronomy of maize and general plant nutrition and fertiliser use with maize as an example. Following an introductory chapter on the origins, economic importance and botanical characteristics of this crop come several chapters discussing, in some detail, methods for maize production. These include sections on cropping systems, rotations, tillage practice, planting, control of weeds, pests and diseases, irrigation and harvesting.

The author reviews the most recent developments in these—for example, minimum tillage and use of herbicides—and draws together information from many parts of the world in discussing their impact on maize growing. A brief chapter on the general nutritional requirements of maize leads to a treatment of the nutrient supplying power of soil and the dynamics of nutrient uptake that would not be out of place in a soil science textbook.

The author is to be congratulated on the clear way in which relatively new concepts of the plant-root system are presented. As the author points out in the foreword, "more fertilisers are probably used for maize than for any other annual crop" and with "its high yield potential, the maize plant has been subjected to more research than most other crop plants". The results of this research have been excellently compiled in chapters dealing with the effects of fertilisers on yields and crop quality, on the important matter of interactions between nutrient elements and on practical aspects of fertiliser use such as soil testing, choice of fertilisers and methods and timing of applications.

Particular attention is paid to the relationship between fertiliser use and the local environment, management practices and maize varieties. Although concentrating on maize, these chapters deal with basic principles of fertiliser use which are relevant to many other crops and situations, making the book valuable even to those not directly interested in maize itself.

The entire book is a fine blend of scientific principles, research findings and practical aspects of crop production that should make it a welcome addition to the bookshelves of anyone

whose interest is in agricultural development. It is well produced, with many graphs, diagrams and tables, and several photographs including some in colour. The only criticism is the lack of an index, or even a list of illustrations, but the overall planning of the chapters makes it relatively easy to find the information required.

Stuart G. McRae

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Phosphorus chemistry

The Chemistry of Phosphorus: Environmental, Organic, Inorganic, Biochemical and Spectroscopic Aspects. By John Emsley and Dennis Hall. Pp. xi+563. (Harper and Row: London and New York, July 1976.) £22.50; \$39.50.

SINCE the appearance about fifteen years ago of the monumental two-volume treatise by Van Wazer on *Phosphorus and its Compounds*, there has been a veritable explosion of monographs and series devoted to various aspect of this important element. Sasse, Hudson, Mann, Corbridge, Allcock, Kosolapoff and Maier (seven edited volumes); *Topics in Phosphorus Chemistry*, and *Organophosphorus Chemistry* (Annual Specialists Reports by the Chemical Society) are just a few of these solely devoted to various aspects of this subject.

Phosphorus chemistry in all its branches—biochemical, organic, inorganic, pure and applied—has made very great strides. Even so, one must closely scrutinise the appearance of a new book, particularly in a field in which so many authors with an international reputation have already put pen to paper. This is particularly pertinent at a time, when one has annually the unpleasant task of deciding which journal subscription has to be cancelled and to choose the very few books (from a long list) the library can afford to purchase (most monographs have already priced themselves out of the market for the individual buyer). As a contributor to Kosolapoff and Maier, I am aware of the pitifully small number of volumes of the standard reference treatise in an important field, which have been sold.

Professor Denney, in the preface, mentions that this is the first book attempting to bring together the various aspects of phosphorus chemistry under one cover. This is indeed the case. But for whom is this book in-

tended? Even a devotee of the subject like myself would hesitate to recommend a book of over 500 pages to undergraduates; it is an important and fascinating facet of Chemistry, but so are many others! In any case they could not afford it. The specialist research workers would require considerably more detail than could possibly have been given in a book of this size. A multi-authored multi-volume treatise would be required for them, but at a price at which most libraries would balk.

I found the book easy to read, although traditionalists might object to colloquialisms, such as: "This suggests that what (POPh)₃ loses on the δ swings it gains on the π roundabouts". I have learned something from sections, where my knowledge is cursory; I was disappointed in those the contents of which I know well. Perhaps this is inevitable in a book of this size.

The authors do not state up to which date they have covered the literature; my own impression is up to 1972, with some references either to secondary sources or to some selected research teams beyond that (mainly 1973). This is probably not the authors' fault; there are inordinate delays in the publication processes for both books and journals.

Obviously in a book of this size one cannot please everybody. Westheimer's work on the hydrolysis of cyclic phosphorus esters is covered, but Wolf's work on spiroposphoranes is hardly touched. The section on nuclear magnetic resonance spectra is thin, and no mention is made of Harris' important work on second-order phenomena. The industrially vital phosphates are barely touched; and Corey's work on the Wittig reaction, and Niecke's and Scherer's work on the isolation (of the hitherto only postulated) three-coordinate five-valent phosphorus species receive no mention. Misinterpretations and misquotations of the literature will be found by the expert eye.

In spite of the above criticism, it would be churlish not to acknowledge the tremendous time and effort the authors have expended on their task, and when used with discretion this book will be useful to some readers.

Although I enjoyed reading the book, the lingering doubt remains: whether the treatment of a subject with this scope in mind, would not have been better served as a "Current Awareness" book at a quarter, or even less, of its length and price, which would then have suited undergraduates and the general scientific reader. **R. A. Shaw**

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